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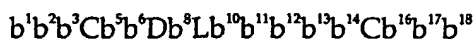
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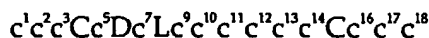
(54) Title: PEPTIDES AND RELATED MOLECULES THAT BIND TO TALL-1



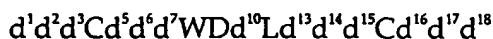
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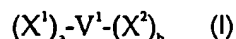


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(SEQ. ID NO: 109)

(57) Abstract: The present invention concerns therapeutic agents that modulate the activity of TALL-1. In accordance with the present invention, modulators of TALL-1 may comprise an amino acid sequence Dz²Lz⁴ wherein z² is an amino acid residue and z⁴ is threonyl or isoleucyl. Exemplary molecules comprise a sequence of the formulae a¹a²a³CDa⁶La⁸a⁹a¹⁰Ca¹²a¹³a¹⁴ (SEQ.ID.NO:100), b¹b²b³Cb⁵b⁶Db⁸Lb¹⁰b¹¹b¹²b¹³b¹⁴Cb¹⁶b¹⁷b¹⁸ (SEQ.ID.NO:104), c¹c²c³Cc⁵Dc⁷Lc⁹c¹⁰c¹¹c¹²c¹³c¹⁴Cc¹⁶c¹⁷c¹⁸ (SEQ.ID.NO:105), d¹d²d³Cd⁵d⁶d⁷WDd¹⁰Ld¹³d¹⁴d¹⁵Cd¹⁶d¹⁷d¹⁸ (SEQ.ID.NO:106), e¹e²e³Ce⁵e⁶e⁷De⁹Le¹¹Ke¹³Ce¹⁵e¹⁶e¹⁷e¹⁸ (SEQ.ID.NO:107), f¹f²f³Kf⁵Df⁷Lf⁹Qf¹²f¹³f¹⁴ (SEQ.ID NO:109) wherein the substituents are as defined in the specification. The invention further comprises compositions of matter of the formula (X¹)-V¹-(X²), wherein V¹ is a vehicle that is covalently attached to one or more of the above TALL-1 modulating compositions of matter. The vehicle and the TALL-1 modulating composition of matter may be linked through the N- or C-terminus of the TALL-1 modulating portion. The preferred vehicle is an Fc domain, and the preferred Fc domain is an IgG Fc domain.



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PEPTIDES AND RELATED MOLECULES THAT BIND TO TALL-1

This application is related to U.S. provisional application no. 60/290,196,
5 filed May 11, 2001, which is hereby incorporated by reference.

Background of the Invention

After years of study in necrosis of tumors, tumor necrosis factors (TNFs) α and β were finally cloned in 1984. The ensuing years witnessed
10 the emergence of a superfamily of TNF cytokines, including fas ligand (FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), CD40 ligand (CD40L), TNF-related apoptosis-inducing ligand (TRAIL, also designated AGP-1), osteoprotegerin binding protein (OPG-BP or OPG ligand), 4-1BB ligand, LIGHT, APRIL, and TALL-1. Smith *et al.* (1994), *Cell* 76: 959-962;
15 Lacey *et al.* (1998), *Cell* 93: 165-176; Chichepotiche *et al.* (1997), *J. Biol. Chem.* 272: 32401-32410; Mauri *et al.* (1998), *Immunity* 8: 21-30; Hahne *et al.* (1998), *J. Exp. Med.* 188: 1185-90; Shu *et al.* (1999), *J. Leukocyte Biology* 65: 680-3. This family is unified by its structure, particularly at the C-terminus. In addition, most members known to date are expressed in
20 immune compartments, although some members are also expressed in other tissues or organs, as well. Smith *et al.* (1994), *Cell* 76: 959-62. All ligand members, with the exception of LT- α , are type II transmembrane proteins, characterized by a conserved 150 amino acid region within C-terminal extracellular domain. Though restricted to only 20-25% identity,
25 the conserved 150 amino acid domain folds into a characteristic β -pleated sheet sandwich and trimerizes. This conserved region can be proteolytically released, thus generating a soluble functional form. Banner *et al.* (1993), *Cell* 73: 431-445.

Many members within this ligand family are expressed in lymphoid enriched tissues and play important roles in the immune system development and modulation. Smith *et al.* (1994). For example, TNF α is mainly synthesized by macrophages and is an important mediator for inflammatory responses and immune defenses. Tracey & Cerami (1994), *Ann. Rev. Med.* 45: 491-503. Fas-L, predominantly expressed in activated T cell, modulates TCR-mediated apoptosis of thymocytes. Nagata, S. & Suda, T. (1995) *Immunology Today* 16: 39-43; Castrim *et al.* (1996), *Immunity* 5: 617-27. CD40L, also expressed by activated T cells, provides an essential signal for B cell survival, proliferation and immunoglobulin isotype switching. Noelle (1996), *Immunity* 4: 415-9.

The cognate receptors for most of the TNF ligand family members have been identified. These receptors share characteristic multiple cysteine-rich repeats within their extracellular domains, and do not possess catalytic motifs within cytoplasmic regions. Smith *et al.* (1994). The receptors signal through direct interactions with death domain proteins (e.g. TRADD, FADD, and RIP) or with the TRAF proteins (e.g. TRAF2, TRAF3, TRAF5, and TRAF6), triggering divergent and overlapping signaling pathways, e.g. apoptosis, NF- κ B activation, or JNK activation. Wallach *et al.* (1999), *Annual Review of Immunology* 17: 331-67. These signaling events lead to cell death, proliferation, activation or differentiation. The expression profile of each receptor member varies. For example, TNFR1 is expressed on a broad spectrum of tissues and cells, whereas the cell surface receptor of OPGL is mainly restricted to the osteoclasts. Hsu *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 3540-5.

A number of research groups have recently identified TNF family ligands with the same or substantially similar sequence. The ligand has been variously named neutrokin α (WO 98/18921, published May 7, 1998), 63954 (WO 98/27114, published June 25, 1998), TL5 (EP 869 180, published October 7, 1998), NTN-2 (WO 98/55620 and WO 98/55621,

published December 10, 1998), TNRL1-alpha (WO 9911791, published March 11, 1999), kay ligand (WO99/12964, published March 18, 1999), and AGP-3 (U.S. Prov. App. Nos. 60/119,906, filed February 12, 1999 and 60/166,271, filed November 18, 1999, respectively); and TALL-1 (WO
5 00/68378, published Nov. 16, 2000). Each of these references is hereby incorporated by reference. Hereinafter, the ligands reported therein are collectively referred to as TALL-1.

TALL-1 is a member of the TNF ligand superfamily that is functionally involved in B cell survival and proliferation. Transgenic mice
10 overexpressing TALL-1 had severe B cell hyperplasia and lupus-like autoimmune disease. Khare *et al.* (2000) *PNAS* 97(7):3370-3375). Both TACI and BCMA serve as cell surface receptors for TALL-1. Gross *et al.* (2000), *Nature* 404: 995-999; Ware (2000), *J. Exp. Med.* 192(11): F35-F37; Ware (2000), *Nature* 404: 949-950; Xia *et al.* (2000), *J. Exp. Med.* 192(1):137-
15 143; Yu *et al.* (2000), *Nature Immunology* 1(3):252-256; Marsters *et al.* (2000), *Current Biology* 10:785-788; Hatzoglou *et al.* (2000) *J. of Immunology* 165:1322-1330; Shu *et al.* (2000) *PNAS* 97(16):9156-9161; Thompson *et al.* (2000) *J. Exp. Med.* 192(1):129-135; Mukhopadhyay *et al.* (1999) *J. Biol. Chem.* 274(23): 15978-81; Shu *et al.* (1999) *J. Leukocyte Biol.*
20 65:680-683; Gruss *et al.* (1995) *Blood* 85(12): 3378-3404; Smith *et al.* (1994), *Cell* 76: 959-962; U.S. Pat. No. 5,969,102, issued October 19, 1999; WO 00/67034, published November 9, 2000; WO 00/40716, published July 13, 2000; WO 99/35170, published July 15, 1999. Both receptors are expressed on B cells and signal through interaction with TRAF proteins. In addition,
25 both TACI and BCMA also bind to another TNF ligand family member, APRIL. Yu *et al.* (2000), *Nature Immunology* 1(3) :252-256. APRIL has also been demonstrated to induce B cell proliferation.

To date, no recombinant or modified proteins employing peptide modulators of TALL-1 have been disclosed. Recombinant and modified

proteins are an emerging class of therapeutic agents. Useful modifications of protein therapeutic agents include combination with the "Fc" domain of an antibody and linkage to polymers such as polyethylene glycol (PEG) and dextran. Such modifications are discussed in detail in a patent
5 application entitled, "Modified Peptides as Therapeutic Agents,"
published WO 00/24782, which is hereby incorporated by reference in its entirety.

A much different approach to development of therapeutic agents is peptide library screening. The interaction of a protein ligand with its
10 receptor often takes place at a relatively large interface. However, as demonstrated for human growth hormone and its receptor, only a few key residues at the interface contribute to most of the binding energy. Clackson *et al.* (1995), *Science* 267: 383-6. The bulk of the protein ligand merely displays the binding epitopes in the right topology or serves
15 functions unrelated to binding. Thus, molecules of only "peptide" length (2 to 40 amino acids) can bind to the receptor protein of a given large protein ligand. Such peptides may mimic the bioactivity of the large protein ligand ("peptide agonists") or, through competitive binding, inhibit the bioactivity of the large protein ligand ("peptide antagonists").

20 Phage display peptide libraries have emerged as a powerful method in identifying such peptide agonists and antagonists. See, for example, Scott *et al.* (1990), *Science* 249: 386; Devlin *et al.* (1990), *Science* 249: 404; U.S. Pat. No. 5,223,409, issued June 29, 1993; U.S. Pat. No. 5,733,731, issued March 31, 1998; U.S. Pat. No. 5,498,530, issued March 12,
25 1996; U.S. Pat. No. 5,432,018, issued July 11, 1995; U.S. Pat. No. 5,338,665, issued August 16, 1994; U.S. Pat. No. 5,922,545, issued July 13, 1999; WO 96/40987, published December 19, 1996; and WO 98/15833, published April 16, 1998 (each of which is incorporated by reference in its entirety). In such libraries, random peptide sequences are displayed by fusion with

coat proteins of filamentous phage. Typically, the displayed peptides are affinity-eluted against an immobilized target protein. The retained phages may be enriched by successive rounds of affinity purification and repropagation. The best binding peptides may be sequenced to identify
5 key residues within one or more structurally related families of peptides. See, e.g., Cwirla *et al.* (1997), *Science* 276: 1696-9, in which two distinct families were identified. The peptide sequences may also suggest which residues may be safely replaced by alanine scanning or by mutagenesis at the DNA level. Mutagenesis libraries may be created and screened to
10 further optimize the sequence of the best binders. Lowman (1997), *Ann. Rev. Biophys. Biomol. Struct.* 26: 401-24.

Structural analysis of protein-protein interaction may also be used to suggest peptides that mimic the binding activity of large protein ligands. In such an analysis, the crystal structure may suggest the identity
15 and relative orientation of critical residues of the large protein ligand, from which a peptide may be designed. See, e.g., Takasaki *et al.* (1997), *Nature Biotech.* 15: 1266-70. These analytical methods may also be used to investigate the interaction between a receptor protein and peptides selected by phage display, which may suggest further modification of the
20 peptides to increase binding affinity.

Other methods compete with phage display in peptide research. A peptide library can be fused to the carboxyl terminus of the *lac* repressor and expressed in *E. coli*. Another *E. coli*-based method allows display on the cell's outer membrane by fusion with a peptidoglycan-associated
25 lipoprotein (PAL). Hereinafter, these and related methods are collectively referred to as "*E. coli* display." In another method, translation of random RNA is halted prior to ribosome release, resulting in a library of polypeptides with their associated RNA still attached. Hereinafter, this and related methods are collectively referred to as "ribosome display."

Other methods employ peptides linked to RNA; for example, PROfusion technology, Phyllos, Inc. See, for example, Roberts & Szostak (1997), Proc. Natl. Acad. Sci. USA, 94: 12297-303. Hereinafter, this and related methods are collectively referred to as "RNA-peptide screening." Chemically

5 derived peptide libraries have been developed in which peptides are immobilized on stable, non-biological materials, such as polyethylene rods or solvent-permeable resins. Another chemically derived peptide library uses photolithography to scan peptides immobilized on glass slides. Hereinafter, these and related methods are collectively referred to as

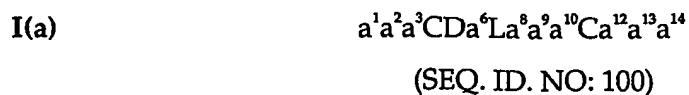
10 "chemical-peptide screening." Chemical-peptide screening may be advantageous in that it allows use of D-amino acids and other unnatural analogues, as well as non-peptide elements. Both biological and chemical methods are reviewed in Wells & Lowman (1992), Curr. Opin. Biotechnol. 3: 355-62. Conceptually, one may discover peptide mimetics of any

15 protein using phage display, RNA-peptide screening, and the other methods mentioned above.

Summary of the Invention

The present invention concerns therapeutic agents that modulate the activity of TALL-1. In accordance with the present invention,

20 modulators of TALL-1 may comprise an amino acid sequence Dz²Lz⁴ (SEQ ID NO: 108) wherein z² is an amino acid residue and z⁴ is threonyl or isoleucyl. Such modulators of TALL-1 comprise molecules of the following formulae:



wherein:

a¹, a², a³ are each independently absent or amino acid residues;

a⁶ is an amino acid residue;

a⁹ is a basic or hydrophobic residue;

30 a⁸ is threonyl or isoleucyl;

a¹² is a neutral polar residue; and
 a¹³ and a¹⁴ are each independently absent or amino acid residues.

I(b) $b^1 b^2 b^3 Cb^5 b^6 Db^8 Lb^{10} b^{11} b^{12} b^{13} b^{14} Cb^{16} b^{17} b^{18}$

5 (SEQ. ID. NO: 104)

wherein:

b¹ and b² are each independently absent or amino acid residues;
 b³ is an acidic or amide residue;
 b⁵ is an amino acid residue;
 10 b⁶ is an aromatic residue;
 b⁸ is an amino acid residue;
 b¹⁰ is T or I;
 b¹¹ is a basic residue;
 b¹² and b¹³ are each independently amino acid residues;
 15 b¹⁴ is a neutral polar residue; and
 b¹⁶, b¹⁷, and b¹⁸ are each independently absent or amino acid
 residues.

I(c) $c^1 c^2 c^3 Cc^5 Dc^7 Lc^9 c^{10} c^{11} c^{12} c^{13} c^{14} Cc^{16} c^{17} c^{18}$

(SEQ. ID. NO:105)

20 wherein:

c¹, c², and c³ are each independently absent or amino acid residues;
 c⁵ is an amino acid residue;
 c⁷ is an amino acid residue;
 c⁹ is T or I;
 25 c¹⁰ is a basic residue;
 c¹¹ and c¹² are each independently amino acid residues;
 c¹³ is a neutral polar residue;
 c¹⁴ is an amino acid residue;
 c¹⁶ is an amino acid residue;

c¹⁷ is a neutral polar residue; and

c¹⁸ is an amino acid residue or is absent.

I(d) d¹d²d³Cd⁵d⁶d⁷WDd¹⁰Ld¹²d¹³d¹⁴Cd¹⁵d¹⁶d¹⁷

(SEQ. ID. NO: 106)

5 wherein:

d¹, d², and d³ are each independently absent or amino acid residues;

d⁵, d⁶, and d⁷ are each independently amino acid residues;

d¹⁰ is an amino acid residue;

d¹³ is T or I;

10 d¹⁴ is an amino acid residue; and

d¹⁶, d¹⁷, and d¹⁸ are each independently absent or amino acid residues.

I(e) e¹e²e³Ce⁵e⁶e⁷De⁹Le¹¹Ke¹³Ce¹⁵e¹⁶e¹⁷e¹⁸

(SEQ. ID. NO: 107)

15 wherein:

e¹, e², and e³ are each independently absent or amino acid residues;

e⁵, e⁶, e⁷, e⁹, and e¹³ are each independently amino acid residues;

e¹¹ is T or I; and

e¹⁵, e¹⁶, and e¹⁷ are each independently absent or amino acid residues.

20 I(f) f¹f²f³Kf⁵Df⁷Lf⁹f¹⁰Qf¹²f¹³f¹⁴

(SEQ. ID NO: 109)

wherein:

f¹, f², and f³ are absent or are amino acid residues (with one of f¹, f²,
and f³ preferred to be C when one of f¹², f¹³, and f¹⁴ is C);

25 f⁵ is W, Y, or F (W preferred);

f⁷ is an amino acid residue (L preferred);

f⁹ is T or I (T preferred);

f¹⁰ is K, R, or H (K preferred);

f^{12} is C, a neutral polar residue, or a basic residue (W, C, or R preferred);

f^{13} is C, a neutral polar residue or is absent (V preferred); and

5 f^{14} is any amino acid residue or is absent;
provided that only one of f^1 , f^2 , and f^3 may be C, and only one of f^{12} , f^{13} , and f^{14} may be C.

Compounds of formulae I(a) through I(f) above incorporate Dz^2Lz^4 , as well as SEQ ID NO: 63 hereinafter. The sequence of I(f) was derived as a consensus sequence as described in Example 1 hereinbelow. Of
10 compounds within formula I(f), those within the formula

I(f) $f^1f^2f^3KWDF^7L^8KQf^{12}f^{13}f^{14}$
(SEQ ID NO: 125)

are preferred. Compounds falling within formula I(f') include SEQ ID
15 NOS: 32, 58, 60, 62, 63, 66, 67, 69, 70, 114, 115, 122, 123, 124, 147-150, 152-177, 179, 180, 187.

Also in accordance with the present invention are compounds having the consensus motif:

PFPWE
20 (SEQ ID NO: 110)

which also bind TALL-1.

Further in accordance with the present invention are compounds of the formulae:

I(g) $g^1g^2g^3Cg^5PFg^8Wg^{10}Cg^{11}g^{12}g^{13}$
25 (SEQ. ID. NO. 101)

wherein:

g^1 , g^2 and g^3 are each independently absent or amino acid residues;
 g^5 is a neutral polar residue;
 g^8 is a neutral polar residue;
30 g^{10} is an acidic residue;

g^{12} and g^{13} are each independently amino acid residues; and
 g^{14} is absent or is an amino acid residue.

I(h) $h^1 h^2 h^3 C W h^6 h^7 W G h^{10} C h^{12} h^{13} h^{14}$
 (SEQ. ID. NO: 102)

5 wherein:

h^1 , h^2 , and h^3 are each independently absent or amino acid residues;
 h^6 is a hydrophobic residue;
 h^7 is a hydrophobic residue;
 h^{10} is an acidic or polar hydrophobic residue; and
 10 h^{12} , h^{13} , and h^{14} are each independently absent or amino acid residues.

I(i) $i^1 i^2 i^3 C i^5 i^6 i^7 i^8 i^9 i^{10} C i^{12} i^{13} i^{14}$
 (SEQ. ID. NO: 103)

wherein:

i^1 is absent or is an amino acid residue;
 15 i^2 is a neutral polar residue;
 i^3 is an amino acid residue;
 i^5 , i^6 , i^7 , and i^8 are each independently amino acid residues;
 i^9 is an acidic residue;
 i^{10} is an amino acid residue;
 20 i^{12} and i^{13} are each independently amino acid residues; and
 i^{14} is a neutral polar residue.

The compounds defined by formulae I(g) through I(i) also bind
 TALL-1.

Further in accordance with the present invention, modulators of
 25 TALL-1 comprise:

a) a TALL-1 modulating domain (e.g., an amino acid sequence
 of Formulae I(a) through I(i)), preferably the amino acid
 sequence Dz^2Lz^4 , or sequences derived therefrom by phage
 display, RNA-peptide screening, or the other techniques
 30 mentioned above; and

- b) a vehicle, such as a polymer (e.g., PEG or dextran) or an Fc domain, which is preferred;

wherein the vehicle is covalently attached to the TALL-1 modulating domain. The vehicle and the TALL-1 modulating domain may be linked through the N- or C-terminus of the TALL-1 modulating domain, as described further below. The preferred vehicle is an Fc domain, and the preferred Fc domain is an IgG Fc domain. Such Fc-linked peptides are referred to herein as "peptibodies." Preferred TALL-1 modulating domains comprise the amino acid sequences described hereinafter in Tables 1 and 2. Other TALL-1 modulating domains can be generated by phage display, RNA-peptide screening and the other techniques mentioned herein.

Further in accordance with the present invention is a process for making TALL-1 modulators, which comprises:

- a. selecting at least one peptide that binds to TALL-1 ; and
b. covalently linking said peptide to a vehicle.

The preferred vehicle is an Fc domain. Step (a) is preferably carried out by selection from the peptide sequences in Table 2 hereinafter or from phage display, RNA-peptide screening, or the other techniques mentioned herein.

The compounds of this invention may be prepared by standard synthetic methods, recombinant DNA techniques, or any other methods of preparing peptides and fusion proteins. Compounds of this invention that encompass non-peptide portions may be synthesized by standard organic chemistry reactions, in addition to standard peptide chemistry reactions when applicable.

The primary use contemplated for the compounds of this invention is as therapeutic or prophylactic agents. The vehicle-linked peptide may

have activity comparable to—or even greater than—the natural ligand mimicked by the peptide.

The compounds of this invention may be used for therapeutic or prophylactic purposes by formulating them with appropriate pharmaceutical carrier materials and administering an effective amount to a patient, such as a human (or other mammal) in need thereof. Other related aspects are also included in the instant invention.

Numerous additional aspects and advantages of the present invention will become apparent upon consideration of the figures and detailed description of the invention.

Brief Description of the Figures

Figure 1 shows exemplary Fc dimers that may be derived from an IgG1 antibody. "Fc" in the figure represents any of the Fc variants within the meaning of "Fc domain" herein. "X¹" and "X²" represent peptides or linker-peptide combinations as defined hereinafter. The specific dimers are as follows:

A, D: Single disulfide-bonded dimers. IgG1 antibodies typically have two disulfide bonds at the hinge region of the antibody. The Fc domain in Figures 1A and 1D may be formed by truncation between the two disulfide bond sites or by substitution of a cysteinyl residue with an unreactive residue (e.g., alanyl). In Figure 1A, the Fc domain is linked at the amino terminus of the peptides; in 1D, at the carboxyl terminus.

B, E: Doubly disulfide-bonded dimers. This Fc domain may be formed by truncation of the parent antibody to retain both cysteinyl residues in the Fc domain chains or by expression from a construct including a sequence encoding such an Fc domain. In Figure 1B, the Fc domain is linked at the amino terminus of the peptides; in 1E, at the carboxyl terminus.

C, F: Noncovalent dimers. This Fc domain may be formed by elimination of the cysteinyl residues by either truncation or substitution. One may desire to eliminate the cysteinyl residues to avoid impurities formed by reaction of the cysteinyl residue with cysteinyl residues of other proteins present in the host cell. The noncovalent bonding of the Fc domains is sufficient to hold together the dimer. Other dimers may be formed by using Fc domains derived from different types of antibodies (e.g., IgG2, IgM).

Figure 2 shows the structure of preferred compounds of the invention that feature tandem repeats of the pharmacologically active peptide. Figure 2A shows a single chain molecule and may also represent the DNA construct for the molecule. Figure 2B shows a dimer in which the linker-peptide portion is present on only one chain of the dimer. Figure 2C shows a dimer having the peptide portion on both chains. The dimer of Figure 2C will form spontaneously in certain host cells upon expression of a DNA construct encoding the single chain shown in Figure 3A. In other host cells, the cells could be placed in conditions favoring formation of dimers or the dimers can be formed in vitro.

Figure 3 shows exemplary nucleic acid and amino acid sequences (SEQ ID NOS: 1 and 2, respectively) of human IgG1 Fc that may be used in this invention.

Figures 4A through 4F show the nucleotide and amino acid sequences (SEQ ID NOS: 3-27) S of NdeI to SalI fragments encoding peptide and linker.

Figures 5A through 5M show the nucleotide sequence (SEQ ID NO: 28) of pAMG21-RANK-Fc vector, which was used to construct Fc-linked molecules of the present invention. These figures identify a number of features of the nucleic acid, including:

- promoter regions PcopB, PrepA, RNAI, APHII, luxPR, and luxPL;
- mRNA for APHII, luxR;

- coding sequences and amino acid sequences for the proteins copB protein, copT, repAI, repA4, APHII, luxR, RANK, and Fc;
- binding sites for the proteins copB, CRP;
- hairpins T1, T2, T7, and toop;
- 5 • operator site for lux protein;
- enzyme restriction sites for PfIII108I, BglII, ScaI, BmnI, DrdII, DraIII, BstBI, AceIII, AflII, PfIMI, BglI, SfiI, BstEII, BspLullI, NspV, BpII, EagI, BcgI, NsiI, BsaI, PspI406I, AatII, BsmI, NruI, NdeI, ApaLI, Acc65I, KpnI, SalI, AccI, BspEI, AhdI, BspHI, EconI, BsrGI, BmaI, SmaI, SexAI, BamHI, and BlpI.

10 Figures 6A and 6B show the DNA sequence (SEQ ID NO: 97) inserted into pCFM1656 between the unique AatII (position #4364 in pCFM1656) and SacII (position #4585 in pCFM1656) restriction sites to form expression plasmid pAMG21 (ATCC accession no. 98113).

Figure 7 shows that the TALL-1 peptibody (SEQ ID NO: 70) inhibits
 15 TALL-1-mediated B cell proliferation. Purified B cells (10^5) from B6 mice were cultured in triplicates in 96-well plated with the indicated amounts of TALL-1 consensus peptibody in the presence of 10 ng/ml TALL-1 plus 2 μ g/ml anti-IgM antibody. Proliferation was measured by radioactive [3 H]thymidine uptake in the last 18h of pulse. Data shown represent mean \pm SD triplicate wells.

20 Figure 8 shows that a TALL-1 N-terminal tandem dimer peptibodies (SEQ ID NO: 123, 124 in Table 5B hereinafter) are preferable for inhibition of TALL-1-mediated B cell proliferation. Purified B cells (10^5) from B6 mice were cultured in triplicates in 96-well plated with the indicated amounts of TALL-1 12-3 peptibody and TALL-1 consensus peptibody (SEQ ID NOS: 115 and 122 of Table 5B) or the
 25 related dimer peptibodies (SEQ ID NOS: 123, 124) in the presence of 10 ng/ml TALL-1 plus 2 μ g/ml anti-IgM antibody. Proliferation was measured by radioactive [3 H]thymidine uptake in the last 18h of pulse. Data shown represent mean \pm SD triplicate wells.

Figure 9. AGP3 peptibody binds to AGP3 with high affinity.
 30 Dissociation equilibrium constant (K_D) was obtained from nonlinear regression

of the competition curves using a dual-curve one-site homogeneous binding model (KinEx™ software). K_D is about 4 pM for AGP3 peptibody binding with human AGP3 (SEQ ID NO: 123).

Figures 10A and 10B. AGP3 peptibody blocks both human and
5 murine AGP3 in the Biacore competition assay. Soluble human TACI protein was immobilized to B1 chip. 1 nM of recombinant human AGP3 protein (upper panel) or 5 nM of recombinant murine AGP3 protein (lower panel) was incubated with indicated amount of AGP3 peptibody before injected over the surface of receptor. Relative human AGP3 and murine AGP3 (binding response was shown
10 (SEQ ID NO: 123).

Figures 11A and 11B. AGP3 peptibody blocked AGP3 binding to all three receptors TACI, BCMA and BAFFR in Biacore competition assay. Recombinant soluble receptor TACI, BCMA and BAFFR proteins were immobilized to CM5 chip. 1 nM of recombinant human AGP3 (upper panel) were
15 incubated with indicated amount of AGP3 peptibody before injected over each receptor surface. Relative binding of AGP3 was measured. Similarly, 1 nM of recombinant APRIL protein was incubated with indicated amount of AGP3 peptibody before injected over each receptor surface. AGP3 peptibody didn't inhibit APRIL binding to all three receptors (SEQ ID NO: 123).

Figures 12A and 12B. AGP3 peptibody inhibits mouse serum
20 immunoglobulin level increase induced by human AGP3 challenge. Balb/c mice received 7 daily intraperitoneal injections of 1 mg/Kg human AGP3 protein along with saline, human Fc, or AGP3 peptibody at indicated doses, and were bled on day 8. Serum total IgM and IgA level were measured by ELISA (SEQ ID NO:
25 123).

Figure 13. AGP3 peptibody treatment reduced arthritis severity in the mouse CIA model. Eight to 12 weeks old DBA/1 male mice were immunized with bovine collagen type II (bCII) emulsified in complete freunds adjuvant intradermally at the base of tail, and were boosted 3 weeks after the initial
30 immunization with bCII emulsified in incomplete freunds adjuvant. Treatment with indicated dosage of AGP3 peptibody was begun from the day of booster

immunization for 4 weeks. As described before (Khare et al., *J. Immunol.* 155: 3653-9, 1995), all four paws were individually scored from 0-3 for arthritis severity (SEQ ID NO: 123).

Figure 14. AGP3 peptibody treatment inhibited anti-collagen antibody
5 generation in the mouse CIA model. Serum samples were taken one week after final treatment (day 35) as described above. Serum anti-collagen II antibody level was determined by ELISA analysis (SEQ ID NO: 123).

Figures 15A and 15B. AGP3 peptibody treatment delayed proteinuria onset and improved survival in NZB/NZW lupus mice. Five-month-old lupus
10 prone NZBx NZBWF1 mice were treated i.p. 3X/week for 8 weeks with PBS or indicated doses of AGP3 peptibody (SEQ ID NO: 123) or human Fc proteins. Protein in the urine was evaluated monthly throughout the life of the experiment with Albustix reagent strips (Bayer AG).

Figures 16A and 16B show the nucleic acid and amino acid
15 sequences of a preferred TALL-1-binding peptibody (SEQ ID NOS: 189 and 123)

Detailed Description of the Invention

Definition of Terms

20 The terms used throughout this specification are defined as follows, unless otherwise limited in specific instances.

General definitions

The term "comprising" means that a compound may include additional amino acids on either or both of the N- or C- termini of the
25 given sequence. Of course, these additional amino acids should not significantly interfere with the activity of the compound.

Additionally, physiologically acceptable salts of the compounds of this invention are also encompassed herein. The term "physiologically acceptable salts" refers to any salts that are known or later discovered to
30 be pharmaceutically acceptable. Some specific examples are: acetate;

trifluoroacetate; hydrohalides, such as hydrochloride and hydrobromide; sulfate; citrate; tartrate; glycolate; and oxalate.

Amino acids

The term "acidic residue" refers to amino acid residues in D- or L-form having sidechains comprising acidic groups. Exemplary acidic residues include D and E.

The term "amide residue" refers to amino acids in D- or L-form having sidechains comprising amide derivatives of acidic groups. Exemplary residues include N and Q.

10 The term "aromatic residue" refers to amino acid residues in D- or L-form having sidechains comprising aromatic groups. Exemplary aromatic residues include F, Y, and W.

The term "basic residue" refers to amino acid residues in D- or L-form having sidechains comprising basic groups. Exemplary basic residues include H, K, and R.

The term "hydrophilic residue" refers to amino acid residues in D- or L-form having sidechains comprising polar groups. Exemplary hydrophilic residues include C, S, T, N, and Q.

20 The term "nonfunctional residue" refers to amino acid residues in D- or L-form having sidechains that lack acidic, basic, or aromatic groups. Exemplary nonfunctional amino acid residues include M, G, A, V, I, L and norleucine (Nle).

The term "neutral polar residue" refers to amino acid residues in D- or L-form having sidechains that lack basic, acidic, or polar groups. Exemplary neutral polar amino acid residues include A, V, L, I, P, W, M, and F.

The term "polar hydrophobic residue" refers to amino acid residues in D- or L-form having sidechains comprising polar groups. Exemplary polar hydrophobic amino acid residues include T, G, S, Y, C, Q, and N.

The term "hydrophobic residue" refers to amino acid residues in D- or L-form having sidechains that lack basic or acidic groups. Exemplary hydrophobic amino acid residues include A, V, L, I, P, W, M, F, T, G, S, Y, C, Q, and N.

5 Peptides

The term "peptide" refers to molecules of 1 to 40 amino acids, with molecules of 5 to 20 amino acids preferred. Exemplary peptides may comprise the TALL-1 modulating domain of a naturally occurring molecule or comprise randomized sequences.

10 The term "randomized" as used to refer to peptide sequences refers to fully random sequences (e.g., selected by phage display methods or RNA-peptide screening) and sequences in which one or more residues of a naturally occurring molecule is replaced by an amino acid residue not appearing in that position in the naturally occurring molecule. Exemplary
15 methods for identifying peptide sequences include phage display, E. coli display, ribosome display, RNA-peptide screening, chemical screening, and the like.

The term "TALL-1 modulating domain" refers to any amino acid sequence that binds to the TALL-1 and comprises naturally occurring
20 sequences or randomized sequences. Exemplary TALL-1 modulating domains can be identified or derived by phage display or other methods mentioned herein.

The term "TALL-1 antagonist" refers to a molecule that binds to the TALL-1 and increases or decreases one or more assay parameters opposite
25 from the effect on those parameters by full length native TALL-1. Such activity can be determined, for example, by such assays as described in the subsection entitled "Biological activity of AGP-3" in the Materials & Methods section of the patent application entitled, "TNF-RELATED PROTEINS", WO 00/47740, published August 17, 2000.

Vehicles and peptibodies

The term "vehicle" refers to a molecule that prevents degradation
5 and/or increases half-life, reduces toxicity, reduces immunogenicity, or
increases biological activity of a therapeutic protein. Exemplary vehicles
include an Fc domain (which is preferred) as well as a linear polymer (e.g.,
polyethylene glycol (PEG), polylysine, dextran, etc.); a branched-chain
polymer (see, for example, U.S. Patent No. 4,289,872 to Denkenwalter et
10 al., issued September 15, 1981; 5,229,490 to Tam, issued July 20, 1993; WO
93/21259 by Frechet et al., published 28 October 1993); a lipid; a
cholesterol group (such as a steroid); a carbohydrate or oligosaccharide
(e.g., dextran); any natural or synthetic protein, polypeptide or peptide
that binds to a salvage receptor; albumin, including human serum
15 albumin (HSA), leucine zipper domain, and other such proteins and
protein fragments. Vehicles are further described hereinafter.

The term "native Fc" refers to molecule or sequence comprising the
sequence of a non-antigen-binding fragment resulting from digestion of
whole antibody, whether in monomeric or multimeric form. The original
20 immunoglobulin source of the native Fc is preferably of human origin and
may be any of the immunoglobulins, although IgG1 and IgG2 are
preferred. Native Fc's are made up of monomeric polypeptides that may
be linked into dimeric or multimeric forms by covalent (i.e., disulfide
bonds) and non-covalent association. The number of intermolecular
25 disulfide bonds between monomeric subunits of native Fc molecules
ranges from 1 to 4 depending on class (e.g., IgG, IgA, IgE) or subclass (e.g.,
IgG1, IgG2, IgG3, IgA1, IgGA2). One example of a native Fc is a disulfide-
bonded dimer resulting from papain digestion of an IgG (see Ellison et al.

(1982), Nucleic Acids Res. 10: 4071-9). The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage
5 receptor, FcRn. International applications WO 97/34631 (published 25 September 1997) and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference in their entirety. Thus, the term "Fc variant" comprises a molecule or sequence that is humanized from a non-human native Fc.
10 Furthermore, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond
15 formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail hereinafter.

20 The term "Fc domain" encompasses native Fc and Fc variant molecules and sequences as defined above. As with Fc variants and native Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

25 The term "multimer" as applied to Fc domains or molecules comprising Fc domains refers to molecules having two or more polypeptide chains associated covalently, noncovalently, or by both covalent and non-covalent interactions. IgG molecules typically form dimers; IgM, pentamers; IgD, dimers; and IgA, monomers, dimers,

trimers, or tetramers. Multimers may be formed by exploiting the sequence and resulting activity of the native Ig source of the Fc or by derivatizing (as defined below) such a native Fc.

The term "dimer" as applied to Fc domains or molecules
5 comprising Fc domains refers to molecules having two polypeptide chains associated covalently or non-covalently. Thus, exemplary dimers within the scope of this invention are as shown in Figure 1.

The terms "derivatizing" and "derivative" or "derivatized" comprise processes and resulting compounds respectively in which (1) the
10 compound has a cyclic portion; for example, cross-linking between cysteinyl residues within the compound; (2) the compound is cross-linked or has a cross-linking site; for example, the compound has a cysteinyl residue and thus forms cross-linked dimers in culture or *in vivo*; (3) one or more peptidyl linkage is replaced by a non-peptidyl linkage; (4) the N-
15 terminus is replaced by $-NRR^1$, $NRC(O)R^1$, $-NRC(O)OR^1$, $-NRS(O)_2R^1$, $-NHC(O)NHR$, a succinimide group, or substituted or unsubstituted benzyloxycarbonyl-NH-, wherein R and R^1 and the ring substituents are as defined hereinafter; (5) the C-terminus is replaced by $-C(O)R^2$ or $-NR^3R^4$ wherein R^2 , R^3 and R^4 are as defined hereinafter; and (6) compounds in
20 which individual amino acid moieties are modified through treatment with agents capable of reacting with selected side chains or terminal residues. Derivatives are further described hereinafter.

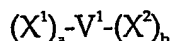
The terms "peptibody" and "peptibodies" refer to molecules comprising an Fc domain and at least one peptide. Such peptibodies may
25 be multimers or dimers or fragments thereof, and they may be derivatized. In the present invention, the molecules of formulae II through VI hereinafter are peptibodies when V^1 is an Fc domain.

Structure of compounds

In General. The present inventors identified sequences capable of binding to and modulating the biological activity of TALL-1. These sequences can be modified through the techniques mentioned above
 5 by which one or more amino acids may be changed while maintaining or even improving the binding affinity of the peptide.

In the compositions of matter prepared in accordance with this invention, the peptide(s) may be attached to the vehicle through the peptide's N-terminus or C-terminus. Any of these peptides may be linked
 10 in tandem (i.e., sequentially), with or without linkers. Thus, the vehicle-peptide molecules of this invention may be described by the following formula:

II



15 wherein:

V^1 is a vehicle (preferably an Fc domain);

X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$, $-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$

P^1 , P^2 , P^3 , and P^4 are each independently sequences of TALL-1
 20 modulating domains, such as those of Formulae I(a) through I(i);

L^1 , L^2 , L^3 , and L^4 are each independently linkers; and

a , b , c , d , e , and f are each independently 0 or 1, provided that at least one of a and b is 1.

Thus, compound II comprises preferred compounds of the
 25 formulae

III



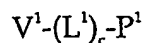
and multimers thereof wherein V^1 is an Fc domain and is attached at the C-terminus of A^1 ;

IV



and multimers thereof wherein V^1 is an Fc domain and is attached at the N-terminus of A^2 ;

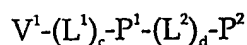
5 V



and multimers thereof wherein V^1 is an Fc domain and is attached at the N-terminus of $-(L^1)_c-P^1$; and

VI

10



and multimers thereof wherein V^1 is an Fc domain and is attached at the N-terminus of $-L^1-P^1-L^2-P^2$.

Peptides. The peptides of this invention are useful as TALL-1 modulating peptides or as TALL-1 modulating domains in the molecules of formulae II through VI. Molecules of this invention comprising these peptide sequences may be prepared by methods known in the art.

Preferred peptide sequences are those of the foregoing formulae I(a) having the substituents identified below.

Table 1--Preferred peptide substituents

Formula I(a)	a^8 is T; a^9 is a basic residue (K most preferred); and a^{12} is a neutral polar residue (F most preferred).
Formula I(b)	b^3 is D, Q, or E; b^6 is W or Y; b^{10} is T; b^{11} is K or R; and b^{14} is V or L.
Formula I(c)	c^9 is T; c^{10} is K or R; c^{13} is a I, L, or V; and c^{17} is A or L.
Formula I(d)	d^{13} is T.
Formula I(e)	e^{11} is T.
Formula I(f)	f^6 is T; f^7 is K; and f^{10} is V.
Formula I(g)	g^5 is W; g^8 is P; g^{10} is E; and g^{13} is a basic residue.
Formula I(h)	h^1 is G; h^6 is A; h^7 is a neutral polar residue; and h^{10} is an acidic residue.
Formula I(i)	i^2 is W; and i^{14} is W.

Preferred peptide sequences appear in Table 2 below.

Table 2—Preferred TALL-1 modulating domains

Sequence	SEQ ID NO:
PGTCFPPFWECTHA	29
WGACWPPFWECFKE	30
VPFCDLLTKHCFEA	31
GSRCYKWDVLTKQCFHH	32
LPGCKWDLLIKQWVCDPL	33
SADCYFDILTKSDVCTSS	34
SDDCMYDQLTRMFICSNL	35
DLNCKYDELTYKEWCQFN	36
FHDCKYDLLTRQMVCHGL	37
RNHCFWDHLLKQDICPSP	38
ANQCWWDLSLTKKNVCEFF	39
YKGRQMWDLILTRSWVSL	126
QDVGLWWDILTRAWMPNI	127
QNAQRVWDLILRTWVYPO	128
GWNEAWWDELTKIWLEQ	129
RITCDTWDSLKKCVPOS	130
GAIMQFWDLSLTKTWLROS	131
WLHSGWWDPLTKHWLQKV	132
SEWFFWFDPLTRAQLKFR	133
GVWFWWFDPLTKQWTQAG	134
MOCKGYDILTKWCVTNG	135
LWSKEVWDILTKSWVSQA	136
KAAGWWFDWLTQVWPAP	137
AYQTFWWDLSLTRLWLSTT	138
SGQHFWDLLTRSWTPST	139
LGVGQKWDPLTKQVSRG	140
VGKMCQWDPLIKRTVCVG	141
CRQGAKFDDLTKQCLLGR	142
GQAIRHWDVLTKQWVDSQ	143
RGPCGSWDLITKHCLDSQ	144
WQWKQWDLITKQMVWVG	145
PITICRKDLLTKQVVCLD	146
KTCNGKWDLLTKQCLQQA	147
KCLKGKWDLLTKQCVTEV	148
RCWNGKWDLLTKQCIHPW	149
NRDMRKWDPLIKQWIVRP	150
QAAAATWDLITKQWLPPP	151
PEGGPKWDPLTKQFLPPV	152
QTPQKKWDLITKQWFTRN	153
IGSPCKWDLITKQMICQT	154
CTAAGKWDLLTKQCIQEK	155
VSQCMKWDLLTKQCLQGW	156
VWGTWKWDLITKQYLPPQ	157
GWWEWKWDLITKQWYRPQ	158
TAQVSKWDLITKQWLPLA	159
QLWGTKWDLITKQYIQIM	160
WATSQKWDLITKQWVQNM	161
QRQCAKWDLITKQCVLFY	162

KT'DCKWDL'LT'KQ'RICQV	163
LLCQ'GKWDL'LT'KQ'CL'KL'R	164
LMWFWKWDL'LT'KQ'LVPT'F	165
Q'TWAWKWDL'LT'KQ'WIGPM	166
NKELLKWDL'LT'KQ'CRGR'S	167
GQKDLKWDL'LT'KQ'YVRQ'S	168
PKPCQKWDL'LT'KQ'CLGS'V	169
GQIGKWDL'LT'KQ'WIQTR	170
VWLDWKWDL'LT'KQ'WIHPQ	171
QEW'EKWDL'LT'KQ'WGLR	172
HWDSWKWDL'LT'KQ'VVQA	173
TRPLQKWDL'LT'KQ'WLRVG	174
SDQWQKWDL'LT'KQ'WFWDV	175
QQTFMKWDL'LT'KQ'WIRRH	176
QGE'CRKWDL'LT'KQ'CFPGQ	177
GQMGWRWDPLIKMCLGPS	178
QLDGCKWDL'LT'KQ'KVCIP	179
HGYWQKWDL'LT'KQ'WVSSE	180
HQ'GQCGWDL'LT'RIYLPCH	181
LHKACKWDL'LT'KQ'CWPMQ	182
GPPGSVWDL'LT'KIWIQTG	183
ITQDWRFD'LT'RLWLPLR	184
QGGFAAWDVLT'KMWITVP	185
GHGT'PWWDALTRI'WILGV	186
VW'PWQKWDL'LT'KQ'VFQD	187
WQWSWKWDL'LT'RQYISS	188
NQTLWKWDL'LT'KQ'FITYM	60
PVYQ'GWWD'LT'KLYIWDG	61
WLDGGWRDPLIKRSVQLG	62
GHQ'QFKWDL'LT'KQ'WVQSN	63
Q'RVGQFWDVLT'KMFITGS	64
QAQ'GWSYDALIKTWIRWP	65
GWMHWKWDP'LT'KQALP'WM	66
GHPTYKWDL'LT'KQ'WILQM	67
WNNWSLWDPLTKLWLQQN	68
WQWGWKWDL'LT'KQ'WVQQQ	69
GOMGWRWDPLTKMWL'GTS	70

It is noted that the known receptors for TALL-1 bear some sequence homology with preferred peptides:

12-3 LPGCKWDLLIKQWVCDPL
5 BAFFR MRRGPRSLRGRDAPVPTPCVPTECYDILLVRKCVDCRILL
TACI TICNHQSQRTCAAFCSRSLSCRKEQGKFYDHLRLRDCISCASI
BCMA FVSPSQEIRGRFRMLQMAQCQSQNEYFDSLHLHACIPCQLRC
(SEQ ID NOS: 33, 195, 196, and 197, respectively).

Any peptide containing a cysteinyl residue may be cross-linked with
10 another Cys-containing peptide, either or both of which may be linked to a

vehicle. Any peptide having more than one Cys residue may form an intrapeptide disulfide bond, as well. Any of these peptides may be derivatized as described hereinafter.

Additional useful peptide sequences may result from conservative
5 and/or non-conservative modifications of the amino acid sequences of the sequences in Table 2.

Conservative modifications will produce peptides having functional and chemical characteristics similar to those of the peptide from which such modifications are made. In contrast, substantial modifications
10 in the functional and/or chemical characteristics of the peptides may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (a) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule
15 at the target site, or (c) the size of the molecule.

For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the
20 polypeptide may also be substituted with alanine, as has been previously described for "alanine scanning mutagenesis" (see, for example, MacLennan et al., 1998, Acta Physiol. Scand. Suppl. 643:55-67; Sasaki et al., 1998, Adv. Biophys. 35:1-24, which discuss alanine scanning mutagenesis).

Desired amino acid substitutions (whether conservative or non-
25 conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the peptide sequence, or to increase or decrease the affinity of the peptide or vehicle-peptide molecules (see preceding formulae) described herein. Exemplary amino acid
30 substitutions are set forth in Table 3.

Table 3—Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

5 In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are

typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

As noted in the foregoing section "Definition of Terms," naturally occurring residues may be divided into classes based on common
5 sidechain properties that may be useful for modifications of sequence. For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the peptide that are homologous with non-human orthologs, or into the non-homologous
10 regions of the molecule. In addition, one may also make modifications using P or G for the purpose of influencing chain orientation.

In making such modifications, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge
15 characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

20 The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte et al., *J. Mol. Biol.*, 157: 105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making
25 changes based upon the hydropathic index, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in the foregoing sequences using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a peptide to similar peptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a peptide that are not conserved relative to such similar peptides would

be less likely to adversely affect the biological activity and/or structure of the peptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the peptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar peptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a peptide that correspond to amino acid residues that are important for activity or structure in similar peptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of the peptides.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a peptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such data could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed,

undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or
5 in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., Curr. Op. in Biotech., 7(4): 422-427 (1996), Chou et al., Biochemistry, 13(2): 222-245 (1974); Chou et al., Biochemistry, 113(2): 211-222 (1974); Chou et al., Adv. Enzymol. Relat.
10 Areas Mol. Biol., 47: 45-148 (1978); Chou et al., Ann. Rev. Biochem., 47: 251-276 and Chou et al., Biophys. J., 26: 367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or
15 proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm et al., Nucl.
20 Acid. Res., 27(1): 244-247 (1999). It has been suggested (Brenner et al., Curr. Op. Struct. Biol., 7(3): 369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

25 Additional methods of predicting secondary structure include "threading" (Jones, D., Curr. Opin. Struct. Biol., 7(3): 377-87 (1997); Sippl et al., Structure, 4(1): 15-9 (1996)), "profile analysis" (Bowie et al., Science, 253: 164-170 (1991); Gribskov et al., Meth. Enzym., 183: 146-159 (1990);

Gribskov et al., Proc. Nat. Acad. Sci., 84(13): 4355-8 (1987)), and "evolutionary linkage" (See Home, supra, and Brenner, supra).

Vehicles. This invention requires the presence of at least one vehicle (V¹) attached to a peptide through the N-terminus, C-terminus or a sidechain of one of the amino acid residues. Multiple vehicles may also be used; e.g., Fc's at each terminus or an Fc at a terminus and a PEG group at the other terminus or a sidechain. Exemplary vehicles include:

- an Fc domain;
- other proteins, polypeptides, or peptides capable of binding to a salvage receptor;
- human serum albumin (HSA);
- a leucine zipper (LZ) domain;
- polyethylene glycol (PEG), including 5 kD, 20 kD, and 30 kD PEG, as well as other polymers;
- dextran;

and other molecules known in the art to provide extended half-life and/or protection from proteolytic degradation or clearance.

An Fc domain is the preferred vehicle. The Fc domain may be fused to the N or C termini of the peptides or at both the N and C termini.

Fusion to the N terminus is preferred.

As noted above, Fc variants are suitable vehicles within the scope of this invention. A native Fc may be extensively modified to form an Fc variant in accordance with this invention, provided binding to the salvage receptor is maintained; see, for example WO 97/34631 and WO 96/32478.

In such Fc variants, one may remove one or more sites of a native Fc that provide structural features or functional activity not required by the fusion molecules of this invention. One may remove these sites by, for example, substituting or deleting residues, inserting residues into the site, or truncating portions containing the site. The inserted or substituted

residues may also be altered amino acids, such as peptidomimetics or D-amino acids. Fc variants may be desirable for a number of reasons, several of which are described below. Exemplary Fc variants include molecules and sequences in which:

- 5 1. Sites involved in disulfide bond formation are removed. Such removal may avoid reaction with other cysteine-containing proteins present in the host cell used to produce the molecules of the invention. For this purpose, the cysteine-containing segment at the N-terminus may be truncated or cysteine residues may be deleted or substituted with other
10 amino acids (e.g., alanyl, seryl). In particular, one may truncate the N-terminal 20-amino acid segment of SEQ ID NO: 2 or delete or substitute the cysteine residues at positions 7 and 10 of SEQ ID NO: 2. Even when cysteine residues are removed, the single chain Fc domains can still form a dimeric Fc domain that is held together non-covalently.
- 15 2. A native Fc is modified to make it more compatible with a selected host cell. For example, one may remove the PA sequence near the N-terminus of a typical native Fc, which may be recognized by a digestive enzyme in E. coli such as proline iminopeptidase. One may also add an N-terminal methionine residue, especially when the molecule is
20 expressed recombinantly in a bacterial cell such as E. coli. The Fc domain of SEQ ID NO: 2 is one such Fc variant.
3. A portion of the N-terminus of a native Fc is removed to prevent N-terminal heterogeneity when expressed in a selected host cell. For this purpose, one may delete any of the first 20 amino acid residues at the
25 N-terminus, particularly those at positions 1, 2, 3, 4 and 5.
4. One or more glycosylation sites are removed. Residues that are typically glycosylated (e.g., asparagine) may confer cytolytic response. Such residues may be deleted or substituted with unglycosylated residues (e.g., alanine).

- 5 5. Sites involved in interaction with complement, such as the C1q binding site, are removed. For example, one may delete or substitute the EKK sequence of human IgG1. Complement recruitment may not be advantageous for the molecules of this invention and so may be avoided with such an Fc variant.
6. Sites are removed that affect binding to Fc receptors other than a salvage receptor. A native Fc may have sites for interaction with certain white blood cells that are not required for the fusion molecules of the present invention and so may be removed.
- 10 7. The ADCC site is removed. ADCC sites are known in the art; see, for example, Molec. Immunol. 29 (5): 633-9 (1992) with regard to ADCC sites in IgG1. These sites, as well, are not required for the fusion molecules of the present invention and so may be removed.
- 15 8. When the native Fc is derived from a non-human antibody, the native Fc may be humanized. Typically, to humanize a native Fc, one will substitute selected residues in the non-human native Fc with residues that are normally found in human native Fc. Techniques for antibody humanization are well known in the art.

Preferred Fc variants include the following. In SEQ ID NO: 2
20 (Figure 3), the leucine at position 15 may be substituted with glutamate; the glutamate at position 99, with alanine; and the lysines at positions 101 and 103, with alanines. In addition, one or more tyrosine residues can be replaced by phenylalanine residues.

An alternative vehicle would be a protein, polypeptide, peptide,
25 antibody, antibody fragment, or small molecule (e.g., a peptidomimetic compound) capable of binding to a salvage receptor. For example, one could use as a vehicle a polypeptide as described in U.S. Pat. No. 5,739,277, issued April 14, 1998 to Presta et al. Peptides could also be selected by phage display or RNA-peptide screening for binding to the

FcRn salvage receptor. Such salvage receptor-binding compounds are also included within the meaning of "vehicle" and are within the scope of this invention. Such vehicles should be selected for increased half-life (e.g., by avoiding sequences recognized by proteases) and decreased
5 immunogenicity (e.g., by favoring non-immunogenic sequences, as discovered in antibody humanization).

As noted above, polymer vehicles may also be used for V¹. Various means for attaching chemical moieties useful as vehicles are currently available, see, e.g., Patent Cooperation Treaty ("PCT") International
10 Publication No. WO 96/11953, entitled "N-Terminally Chemically Modified Protein Compositions and Methods," herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water soluble polymers to the N-terminus of proteins.

15 A preferred polymer vehicle is polyethylene glycol (PEG). The PEG group may be of any convenient molecular weight and may be linear or branched. The average molecular weight of the PEG will preferably range from about 2 kiloDalton ("kD") to about 100 kD, more preferably from about 5 kD to about 50 kD, most preferably from about 5 kD to about 10
20 kD. The PEG groups will generally be attached to the compounds of the invention via acylation or reductive alkylation through a reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

25 A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are "preactivated" with

an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

Polysaccharide polymers are another type of water soluble polymer which may be used for protein modification. Dextran is a polysaccharide polymer comprised of individual subunits of glucose predominantly linked by α 1-6 linkages. The dextran itself is available in many molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water soluble polymer for use in the present invention as a vehicle by itself or in combination with another vehicle (e.g., Fc). See, for example, WO 96/11953 and WO 96/05309. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported; see, for example, European Patent Publication No. 0 315 456, which is hereby incorporated by reference in its entirety. Dextran of about 1 kD to about 20 kD is preferred when dextran is used as a vehicle in accordance with the present invention.

Linkers. Any "linker" group is optional. When present, its chemical structure is not critical, since it serves primarily as a spacer. The linker is preferably made up of amino acids linked together by peptide bonds. Thus, in preferred embodiments, the linker is made up of from 1 to 30 amino acids linked by peptide bonds, wherein the amino acids are selected from the 20 naturally occurring amino acids. Some of these amino acids may be glycosylated, as is well understood by those in the art. In a more preferred embodiment, the 1 to 20 amino acids are selected from glycine, alanine, proline, asparagine, glutamine, and lysine. Even more preferably,

a linker is made up of a majority of amino acids that are sterically unhindered, such as glycine and alanine. Thus, preferred linkers are polyglycines (particularly (Gly)₄, (Gly)₅), poly(Gly-Ala), and polyalanines. Other specific examples of linkers are:

- 5 (Gly)₃Lys(Gly)₄ (SEQ ID NO: 40);
 (Gly)₃AsnGlySer(Gly)₂ (SEQ ID NO: 41);
 (Gly)₃Cys(Gly)₄ (SEQ ID NO: 42); and
 GlyProAsnGlyGly (SEQ ID NO: 43).

To explain the above nomenclature, for example, (Gly)₃Lys(Gly)₄ means
 10 Gly-Gly-Gly-Lys-Gly-Gly-Gly-Gly (SEQ ID NO: 40). Combinations of Gly and Ala are also preferred. The linkers shown here are exemplary; linkers within the scope of this invention may be much longer and may include other residues.

Preferred linkers are amino acid linkers comprising greater than 5
 15 amino acids, with suitable linkers having up to about 500 amino acids selected from glycine, alanine, proline, asparagine, glutamine, lysine, threonine, serine or aspartate. Linkers of about 20 to 50 amino acids are most preferred. One group of preferred linkers are those of the formulae

20 GSGSATGGSGSTASSGSGSATx¹x²
 (SEQ ID NO: 193)

and

GSGSATGGSGSTASSGSGSATx¹x²GSGSATGGSGSTASSGSGSATx³x⁴
 (SEQ ID NO: 194)

wherein x¹ and x³ are each independently basic or hydrophobic residues
 25 and x² and x⁴ are each independently hydrophobic residues. Specific preferred linkers are:

GSGSATGGSGSTASSGSGSATHM
 (SEQ ID NO: 59)

GSGSATGGSGSTASSGSGSATGM

(SEQ ID NO: 190)

GSGSATGGSGSTASSGSGSATGS

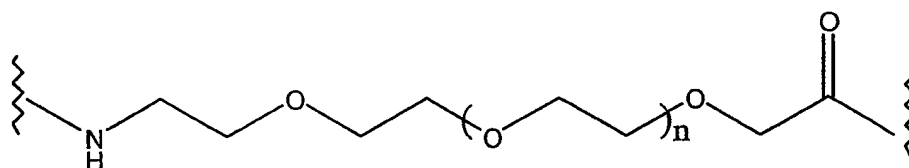
(SEQ ID NO: 191), and

5 GSGSATGGSGSTASSGSGSATHMGSGSATGGSGSTASSGSGSATHM

(SEQ ID NO: 192).

Non-peptide linkers are also possible. For example, alkyl linkers such as $-\text{NH}-(\text{CH}_2)_s-\text{C}(\text{O})-$, wherein $s = 2-20$ could be used. These alkyl linkers may further be substituted by any non-sterically hindering group
10 such as lower alkyl (e.g., C_1-C_6) lower acyl, halogen (e.g., Cl, Br), CN, NH_2 , phenyl, etc. An exemplary non-peptide linker is a PEG linker,

VII



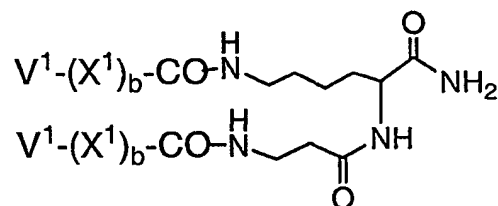
15 wherein n is such that the linker has a molecular weight of 100 to 5000 kD, preferably 100 to 500 kD. The peptide linkers may be altered to form derivatives in the same manner as described above.

Derivatives. The inventors also contemplate derivatizing the peptide and/or vehicle portion of the compounds. Such derivatives may
20 improve the solubility, absorption, biological half life, and the like of the compounds. The moieties may alternatively eliminate or attenuate any undesirable side-effect of the compounds and the like. Exemplary derivatives include compounds in which:

1. The compound or some portion thereof is cyclic. For example, the
25 peptide portion may be modified to contain two or more Cys residues (e.g., in the linker), which could cyclize by disulfide bond formation.

2. The compound is cross-linked or is rendered capable of cross-linking between molecules. For example, the peptide portion may be modified to contain one Cys residue and thereby be able to form an intermolecular disulfide bond with a like molecule. The compound may also be cross-linked through its C-terminus, as in the molecule shown below.

VIII



In Formula VIII, each "V¹" may represent typically one strand of the Fc domain.

3. One or more peptidyl [-C(O)NR-] linkages (bonds) is replaced by a non-peptidyl linkage. Exemplary non-peptidyl linkages are -CH₂-carbamate [-CH₂-OC(O)NR-], phosphonate, -CH₂-sulfonamide [-CH₂-S(O)₂NR-], urea [-NHC(O)NH-], -CH₂-secondary amine, and alkylated peptide [-C(O)NR⁶- wherein R⁶ is lower alkyl].
4. The N-terminus is derivatized. Typically, the N-terminus may be acylated or modified to a substituted amine. Exemplary N-terminal derivative groups include -NRR¹ (other than -NH₂), -NRC(O)R¹, -NRC(O)OR¹, -NRS(O)₂R¹, -NHC(O)NHR¹, succinimide, or benzyloxycarbonyl-NH- (CBZ-NH-), wherein R and R¹ are each independently hydrogen or lower alkyl and wherein the phenyl ring may be substituted with 1 to 3 substituents selected from the group consisting of C₁-C₄ alkyl, C₁-C₄ alkoxy, chloro, and bromo.
5. The free C-terminus is derivatized. Typically, the C-terminus is esterified or amidated. Exemplary C-terminal derivative groups include, for example, -C(O)R² wherein R² is lower alkoxy or -NR³R⁴

wherein R³ and R⁴ are independently hydrogen or C₁-C₈ alkyl (preferably C₁-C₄ alkyl).

6. A disulfide bond is replaced with another, preferably more stable, cross-linking moiety (e.g., an alkylene). See, e.g., Bhatnagar *et al.* (1996), *J. Med. Chem.* 39: 3814-9; Alberts *et al.* (1993) *Thirteenth Am. Pep. Symp.*, 357-9.
7. One or more individual amino acid residues is modified. Various derivatizing agents are known to react specifically with selected sidechains or terminal residues, as described in detail below.

10 Lysinyl residues and amino terminal residues may be reacted with succinic or other carboxylic acid anhydrides, which reverse the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic
15 acid; O-methylisourea; 2,4 pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues may be modified by reaction with any one or combination of several conventional reagents, including phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginyl
20 residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

Specific modification of tyrosyl residues has been studied extensively,
25 with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively.

Carboxyl sidechain groups (aspartyl or glutamyl) may be selectively modified by reaction with carbodiimides ($R'-N=C=N-R'$) such as 1-cyclohexyl-3-(2-morpholinyl-(4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues
5 may be converted to asparaginyll and glutaminyll residues by reaction with ammonium ions.

Glutaminyll and asparaginyll residues may be deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues
10 falls within the scope of this invention.

Cysteinyl residues can be replaced by amino acid residues or other moieties either to eliminate disulfide bonding or, conversely, to stabilize cross-linking. See, e.g., Bhatnagar *et al.* (1996), *J. Med. Chem.* 39: 3814-9.

Derivatization with bifunctional agents is useful for cross-linking the
15 peptides or their functional derivatives to a water-insoluble support matrix or to other macromolecular vehicles. Commonly used cross-linking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-
20 dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimide yield photoactivatable intermediates that are capable of forming cross-links in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates
25 and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Carbohydrate (oligosaccharide) groups may conveniently be attached to sites that are known to be glycosylation sites in proteins.

Generally, O-linked oligosaccharides are attached to serine (Ser) or threonine (Thr) residues while N-linked oligosaccharides are attached to asparagine (Asn) residues when they are part of the sequence Asn-X-Ser/Thr, where X can be any amino acid except proline. X is preferably one of the 19 naturally occurring amino acids other than proline. The structures of N-linked and O-linked oligosaccharides and the sugar residues found in each type are different. One type of sugar that is commonly found on both is N-acetylneuraminic acid (referred to as sialic acid). Sialic acid is usually the terminal residue of both N-linked and O-linked oligosaccharides and, by virtue of its negative charge, may confer acidic properties to the glycosylated compound. Such site(s) may be incorporated in the linker of the compounds of this invention and are preferably glycosylated by a cell during recombinant production of the polypeptide compounds (e.g., in mammalian cells such as CHO, BHK, COS). However, such sites may further be glycosylated by synthetic or semi-synthetic procedures known in the art.

Other possible modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, oxidation of the sulfur atom in Cys, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains. Creighton, Proteins: Structure and Molecule Properties (W. H. Freeman & Co., San Francisco), pp. 79-86 (1983).

Compounds of the present invention may be changed at the DNA level, as well. The DNA sequence of any portion of the compound may be changed to codons more compatible with the chosen host cell. For E. coli, which is the preferred host cell, optimized codons are known in the art. Codons may be substituted to eliminate restriction sites or to include silent restriction sites, which may aid in processing of the DNA in the selected

host cell. The vehicle, linker and peptide DNA sequences may be modified to include any of the foregoing sequence changes.

Methods of Making

The compounds of this invention largely may be made in transformed host cells using recombinant DNA techniques. To do so, a
5 recombinant DNA molecule coding for the peptide is prepared. Methods of preparing such DNA molecules are well known in the art. For instance, sequences coding for the peptides could be excised from DNA using suitable restriction enzymes. Alternatively, the DNA molecule could be
10 synthesized using chemical synthesis techniques, such as the phosphoramidate method. Also, a combination of these techniques could be used.

The invention also includes a vector capable of expressing the peptides in an appropriate host. The vector comprises the DNA molecule
15 that codes for the peptides operatively linked to appropriate expression control sequences. Methods of effecting this operative linking, either before or after the DNA molecule is inserted into the vector, are well known. Expression control sequences include promoters, activators, enhancers, operators, ribosomal binding sites, start signals, stop signals,
20 cap signals, polyadenylation signals, and other signals involved with the control of transcription or translation.

The resulting vector having the DNA molecule thereon is used to transform an appropriate host. This transformation may be performed using methods well known in the art.

25 Any of a large number of available and well-known host cells may be used in the practice of this invention. The selection of a particular host is dependent upon a number of factors recognized by the art. These include, for example, compatibility with the chosen expression vector, toxicity of the peptides encoded by the DNA molecule, rate of

transformation, ease of recovery of the peptides, expression characteristics, bio-safety and costs. A balance of these factors must be struck with the understanding that not all hosts may be equally effective for the expression of a particular DNA sequence. Within these general guidelines, 5 useful microbial hosts include bacteria (such as E. coli sp.), yeast (such as Saccharomyces sp.) and other fungi, insects, plants, mammalian (including human) cells in culture, or other hosts known in the art.

Next, the transformed host is cultured and purified. Host cells may be cultured under conventional fermentation conditions so that the 10 desired compounds are expressed. Such fermentation conditions are well known in the art. Finally, the peptides are purified from culture by methods well known in the art.

The compounds may also be made by synthetic methods. For example, solid phase synthesis techniques may be used. Suitable 15 techniques are well known in the art, and include those described in Merrifield (1973), Chem. Polypeptides, pp. 335-61 (Katsoyannis and Panayotis eds.); Merrifield (1963), J. Am. Chem. Soc. 85: 2149; Davis et al. (1985), Biochem. Intl. 10: 394-414; Stewart and Young (1969), Solid Phase Peptide Synthesis; U.S. Pat. No. 3,941,763; Finn et al. (1976), The Proteins 20 (3rd ed.) 2: 105-253; and Erickson et al. (1976), The Proteins (3rd ed.) 2: 257-527. Solid phase synthesis is the preferred technique of making individual peptides since it is the most cost-effective method of making small peptides.

Compounds that contain derivatized peptides or which contain 25 non-peptide groups may be synthesized by well-known organic chemistry techniques.

Uses of the Compounds

Compounds of this invention may be particularly useful in treatment of B-cell mediated autoimmune diseases. In particular, the

compounds of this invention may be useful in treating, preventing, ameliorating, diagnosing or prognosing lupus, including systemic lupus erythematosus (SLE), and lupus-associated diseases and conditions. Other preferred indications include B-cell mediated cancers, including B-cell lymphoma.

The compounds of this invention can also be used to treat inflammatory conditions of the joints. Inflammatory conditions of a joint are chronic joint diseases that afflict and disable, to varying degrees, millions of people worldwide. Rheumatoid arthritis is a disease of articular joints in which the cartilage and bone are slowly eroded away by a proliferative, invasive connective tissue called pannus, which is derived from the synovial membrane. The disease may involve peri-articular structures such as bursae, tendon sheaths and tendons as well as extra-articular tissues such as the subcutis, cardiovascular system, lungs, spleen, lymph nodes, skeletal muscles, nervous system (central and peripheral) and eyes (Silberberg (1985), Anderson's Pathology, Kissane (ed.), II:1828). Osteoarthritis is a common joint disease characterized by degenerative changes in articular cartilage and reactive proliferation of bone and cartilage around the joint. Osteoarthritis is a cell-mediated active process that may result from the inappropriate response of chondrocytes to catabolic and anabolic stimuli. Changes in some matrix molecules of articular cartilage reportedly occur in early osteoarthritis (Thonar *et al.* (1993), *Rheumatic disease clinics of North America*, Moskowitz (ed.), 19:635-657 and Shinmei *et al.* (1992), *Arthritis Rheum.*, 35:1304-1308). TALL-1, TALL-1R and modulators thereof are believed to be useful in the treatment of these and related conditions.

Compounds of this invention may also be useful in treatment of a number of additional diseases and disorders, including:

- acute pancreatitis;

- ALS;
- Alzheimer's disease;
- asthma;
- atherosclerosis;
- 5 • autoimmune hemolytic anemia;
- cancer, particularly cancers related to B cells;
- cachexia/anorexia;
- chronic fatigue syndrome;
- cirrhosis (e.g., primary biliary cirrhosis);
- 10 • diabetes (e.g., insulin diabetes);
- fever;
- glomerulonephritis, including IgA glomerulonephritis and
primary glomerulonephritis;
- Goodpasture's syndrome;
- 15 • Guillain-Barre syndrome;
- graft versus host disease;
- Hashimoto's thyroiditis;
- hemorrhagic shock;
- hyperalgesia;
- 20 • inflammatory bowel disease;
- inflammatory conditions of a joint, including osteoarthritis,
psoriatic arthritis and rheumatoid arthritis;
- inflammatory conditions resulting from strain, sprain, cartilage
damage, trauma, orthopedic surgery, infection or other disease
25 processes;
- insulin-dependent diabetes mellitus;

- ischemic injury, including cerebral ischemia (e.g., brain injury as a result of trauma, epilepsy, hemorrhage or stroke, each of which may lead to neurodegeneration);
- learning impairment;
- 5 • lung diseases (e.g., ARDS);
- multiple myeloma;
- multiple sclerosis;
- Myasthenia gravis;
- myelogenous (e.g., AML and CML) and other leukemias;
- 10 • myopathies (e.g., muscle protein metabolism, esp. in sepsis);
- neurotoxicity (e.g., as induced by HIV);
- osteoporosis;
- pain;
- Parkinson's disease;
- 15 • Pemphigus;
- polymyositis/dermatomyositis;
- pulmonary inflammation, including autoimmune pulmonary inflammation;
- pre-term labor;
- 20 • psoriasis;
- Reiter's disease;
- reperfusion injury;
- septic shock;
- side effects from radiation therapy;
- 25 • Sjogren's syndrome;
- sleep disturbance;
- temporal mandibular joint disease;

- thrombocytopenia, including idiopathic thrombocytopenia and autoimmune neonatal thrombocytopenia;
- tumor metastasis;
- uveitis; and
- vasculitis.

5

Compounds of this invention may be administered alone or in combination with a therapeutically effective amount of other drugs, including analgesic agents, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and any
10 immune and/or inflammatory modulators. Thus, compounds of this invention may be administered with:

15

- Modulators of other members of the TNF/TNF receptor family, including TNF antagonists, such as etanercept (Enbrel™), sTNF-RI, onercept, D2E7, and Remicade™.
- Nerve growth factor (NGF) modulators.
- IL-1 inhibitors, including IL-1ra molecules such as anakinra and more recently discovered IL-1ra-like molecules such as IL-1Hy1 and IL-1Hy2; IL-1 "trap" molecules as described in U.S. Pat. No. 5,844,099, issued December 1, 1998; IL-1 antibodies; solubilized
20 IL-1 receptor, and the like.
- IL-6 inhibitors (e.g., antibodies to IL-6).
- IL-8 inhibitors (e.g., antibodies to IL-8).
- IL-18 inhibitors (e.g., IL-18 binding protein, solubilized IL-18 receptor, or IL-18 antibodies).
- Interleukin-1 converting enzyme (ICE) modulators.
- insulin-like growth factors (IGF-1, IGF-2) and modulators thereof.
- Transforming growth factor- β (TGF- β), TGF- β family members, and TGF- β modulators.

25

- Fibroblast growth factors FGF-1 to FGF-10, and FGF modulators.
- Osteoprotegerin (OPG), OPG analogues, osteoprotective agents, and antibodies to OPG-ligand (OPG-L).
- 5 • bone anabolic agents, such as parathyroid hormone (PTH), PTH fragments, and molecules incorporating PTH fragments (e.g., PTH (1-34)-Fc).
- PAF antagonists.
- Keratinocyte growth factor (KGF), KGF-related molecules (e.g.,
10 KGF-2), and KGF modulators.
- COX-2 inhibitors, such as Celebrex™ and Vioxx™.
- Prostaglandin analogs (e.g., E series prostaglandins).
- Matrix metalloproteinase (MMP) modulators.
- Nitric oxide synthase (NOS) modulators, including modulators
15 of inducible NOS.
- Modulators of glucocorticoid receptor.
- Modulators of glutamate receptor.
- Modulators of lipopolysaccharide (LPS) levels.
- Anti-cancer agents, including inhibitors of oncogenes (e.g., fos,
20 jun) and interferons.
- Noradrenaline and modulators and mimetics thereof.

Pharmaceutical Compositions

In General. The present invention also provides methods of using pharmaceutical compositions of the inventive compounds. Such pharmaceutical compositions may be for administration for injection, or for
5 oral, pulmonary, nasal, transdermal or other forms of administration. In general, the invention encompasses pharmaceutical compositions comprising effective amounts of a compound of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various
10 buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of
15 polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's
20 Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference in their entirety. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

25 Oral dosage forms. Contemplated for use herein are oral solid dosage forms, which are described generally in Chapter 89 of Remington's Pharmaceutical Sciences (1990), 18th Ed., Mack Publishing Co. Easton PA 18042, which is herein incorporated by reference in its entirety. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets

or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given in Chapter 10 of Marshall, K., Modern Pharmaceutics (1979), edited by G. S. Banker and C. T. Rhodes, herein incorporated by reference in its entirety. In general, the formulation will include the inventive compound, and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

Also specifically contemplated are oral dosage forms of the above inventive compounds. If necessary, the compounds may be chemically modified so that oral delivery is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the compound molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the compound and increase in circulation time in the body. Moieties useful as covalently attached vehicles in this invention may also be used for this purpose. Examples of such moieties include: PEG, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. See, for example, Abuchowski and Davis, Soluble Polymer-Enzyme Adducts, Enzymes as Drugs (1981), Hocenberg and Roberts, eds., Wiley-Interscience, New York, NY, , pp. 367-83; Newmark, et al. (1982), J. Appl. Biochem. 4:185-9. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are PEG moieties.

For oral delivery dosage forms, it is also possible to use a salt of a modified aliphatic amino acid, such as sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC), as a carrier to enhance absorption of the therapeutic compounds of this invention. The clinical efficacy of a heparin
5 formulation using SNAC has been demonstrated in a Phase II trial conducted by Emisphere Technologies. See US Patent No. 5,792,451, "Oral drug delivery composition and methods".

The compounds of this invention can be included in the formulation as fine multiparticulates in the form of granules or pellets of
10 particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or
15 microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

One may dilute or increase the volume of the compound of the invention with an inert material. These diluents could include
20 carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

25 Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrants include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange

peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or
5 tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl
10 cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants
15 may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of
20 various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

25 To aid dissolution of the compound of this invention into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or

benzethonium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid
5 ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

Additives may also be included in the formulation to enhance uptake of the compound. Additives potentially having this property are
10 for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

Controlled release formulation may be desirable. The compound of this invention could be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms; e.g., gums. Slowly degenerating matrices may also be incorporated into the formulation, e.g.,
15 alginates, polysaccharides. Another form of a controlled release of the compounds of this invention is by a method based on the Oros therapeutic system (Alza Corp.), i.e., the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also
20 have a delayed release effect.

Other coatings may be used for the formulation. These include a variety of sugars which could be applied in a coating pan. The therapeutic agent could also be given in a film coated tablet and the materials used in this instance are divided into 2 groups. The first are the nonenteric
25 materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

Pulmonary delivery forms. Also contemplated herein is pulmonary
5 delivery of the present protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei *et al.*, *Pharma. Res.* (1990) 7: 565-9; Adjei *et al.* (1990), *Internatl. J. Pharmaceutics* 63: 135-44 (leuprolide acetate); Braquet
10 *et al.* (1989), *J. Cardiovasc. Pharmacol.* 13 (suppl.5): s.143-146 (endothelin-1); Hubbard *et al.* (1989), *Annals Int. Med.* 3: 206-12 (α 1-antitrypsin); Smith *et al.* (1989), *J. Clin. Invest.* 84: 1145-6 (α 1-proteinase); Oswein *et al.* (March 1990), "Aerosolization of Proteins", *Proc. Symp. Resp. Drug Delivery II*, Keystone, Colorado (recombinant human growth hormone); Debs *et al.*
15 (1988), *J. Immunol.* 140: 3482-8 (interferon- γ and tumor necrosis factor α) and Platz *et al.*, U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor).

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of
20 therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the
25 Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations suitable for the dispensing of the inventive compound. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to diluents, adjuvants
5 and/or carriers useful in therapy.

The inventive compound should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

10 Pharmaceutically acceptable carriers include carbohydrates such as trehalose, mannitol, xylitol, sucrose, lactose, and sorbitol. Other ingredients for use in formulations may include DPPC, DOPE, DSPC and DOPC. Natural or synthetic surfactants may be used. PEG may be used (even apart from its use in derivatizing the protein or analog).
15 Dextrans, such as cyclodextran, may be used. Bile salts and other related enhancers may be used. Cellulose and cellulose derivatives may be used. Amino acids may be used, such as use in a buffer formulation.

Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

20 Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise the inventive compound dissolved in water at a concentration of about 0.1 to 25 mg of biologically active protein per mL of solution. The formulation may also include a buffer and a simple sugar (e.g., for protein stabilization and regulation of osmotic
25 pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the protein caused by atomization of the solution in forming the aerosol.

Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the inventive

compound suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing the inventive compound and may also include a bulking agent, such as lactose, sorbitol, sucrose, mannitol, trehalose, or xylitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation.

Nasal delivery forms. Nasal delivery of the inventive compound is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung.

Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucous membranes is also contemplated.

Dosages. The dosage regimen involved in a method for treating the above-described conditions will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. Generally, the daily regimen should be in the range of 0.1-1000 micrograms of the inventive compound per kilogram of body weight, preferably 0.1-150 micrograms per kilogram.

Specific preferred embodiments

The inventors have determined preferred structures for the preferred peptides listed in Table 4 below. The symbol "Λ" may be any of the linkers described herein or may simply represent a normal peptide bond (i.e., so that no linker is present). Tandem repeats and linkers are shown separated by dashes for clarity.

Table 4—Preferred embodiments

Sequence/structure	SEQ ID NO:
LPGCKWDLLIKQWVCDPL-Λ-V ¹	44
V ¹ -Λ- LPGCKWDLLIKQWVCDPL	45
LPGCKWDLLIKQWVCDPL -Λ- LPGCKWDLLIKQWVCDPL -Λ-V ¹	46
V ¹ -Λ- LPGCKWDLLIKQWVCDPL -Λ- LPGCKWDLLIKQWVCDPL	47
SADCYFDILTKSDVCTSS-Λ-V ¹	48
V ¹ -Λ- SADCYFDILTKSDVCTSS	49
SADCYFDILTKSDVTSS-Λ- SADCYFDILTKSDVTSS -Λ-V ¹	50
V ¹ -Λ- SADCYFDILTKSDVTSS -Λ- SADCYFDILTKSDVTSS	51
FHDCKWDLLTKQWVCHGL-Λ-V ¹	52
V ¹ -Λ- FHDCKWDLLTKQWVCHGL	53
FHDCKWDLLTKQWVCHGL -Λ- FHDCKWDLLTKQWVCHGL -Λ-V ¹	54
V ¹ -Λ- FHDCKWDLLTKQWVCHGL -Λ- FHDCKWDLLTKQWVCHGL	55

"V¹" is an Fc domain as defined previously herein. In addition to those listed in Table 4, the inventors further contemplate heterodimers in which each strand of an Fc dimer is linked to a different peptide sequence; for example, wherein each Fc is linked to a different sequence selected from Table 2.

All of the compounds of this invention can be prepared by methods described in PCT appl. no. WO 99/25044.

The invention will now be further described by the following working examples, which are illustrative rather than limiting.

EXAMPLE 1

Peptides

5 Peptide Phage Display

1. Magnetic bead preparation

A. Fc-TALL-1 immobilization on magnetic beads

The recombinant Fc-TALL-1 protein was immobilized on the Protein A Dynabeads (Dyna) at a concentration of 8 µg of Fc-TALL-1 per 100 µl of the
10 bead stock from the manufacturer. By drawing the beads to one side of a tube using a magnet and pipetting away the liquid, the beads were washed twice with the phosphate buffer saline (PBS) and resuspended in PBS. The Fc-TALL-1 protein was added to the washed beads at the above concentration and incubated with rotation for 1 hour at room temperature. The Fc-TALL-1 coated beads were
15 then blocked by adding bovine serum albumin (BSA) to 1% final concentration and incubating overnight at 4 °C with rotation. The resulting Fc-TALL-1 coated beads were then washed twice with PBST (PBS with 0.05% Tween-20) before being subjected to the selection procedures.

B. Negative selection bead preparation

20 Additional beads were also prepared for negative selections. For each panning condition, 250 µl of the bead stock from the manufacturer was subjected to the above procedure (section 1A) except that the incubation step with Fc-TALL-1 was omitted. In the last washing step, the beads were divided into five 50 µl aliquots.

25 2. Selection of TALL-1 binding phage

A. Overall strategy

Two filamentous phage libraries, TN8-IX (5×10^9 independent transformants) and TN12-I (1.4×10^9 independent transformants) (Dyax Corp.), were used to select for TALL-1 binding phage. Each library was subjected to
30 either pH 2 elution or 'bead elution' (section 2E). Therefore, four different panning conditions were carried out for the TALL-1 project (TN8-IX using the

pH2 elution method, TN8-IX using the bead elution method, TN12-I the using pH2 elution method, and TN12-I using the bead elution method). Three rounds of selection were performed for each condition.

B. Negative selection

5 For each panning condition, about 100 random library equivalent (5×10^{11} pfu for TN8-IX and 1.4×10^{11} pfu for TN12-I) was aliquoted from the library stock and diluted to 300 μ l with PBST. After the last washing liquid was drawn out from the first 50 μ l aliquot of the beads prepared for negative selections (section 1B), the 300 μ l diluted library stock was added to the beads. The resulting
10 mixture was incubated for 10 minutes at room temperature with rotation. The phage supernatant was drawn out using the magnet and added to the second 50 μ l aliquot for another negative selection step. In this way, five negative selection steps were performed.

C. Selection using the Fc-TALL-1 protein coated beads

15 The phage supernatant after the last negative selection step (section 1B) was added to the Fc-TALL-1 coated beads after the last washing step (section 1A). This mixture was incubated with rotation for two hours at room temperature, allowing specific phage to bind to the target protein. After the supernatant is discarded, the beads were washed seven times with PBST.

20 D. pH2 elution of bound phage

After the last washing step (section 2C), the bound phages were eluted from the magnetic beads by adding 200 μ l of CBST (50 mM sodium citrate, 150 mM sodium chloride, 0.05% Tween-20, pH2). After 5 minute incubation at room temperature, the liquid containing the eluted phage were drawn out and transferred
25 to another tube. The elution step was repeated again by adding 200 μ l of CBST and incubating for 5 minutes. The liquids from two elution steps were added together, and 100 μ l of 2 M Tris solution (pH 8) was added to neutralize the pH. 500 μ l of Min A Salts solution (60 mM K_2HPO_4 , 33 mM KH_2PO_4 , 7.6 mM $(NH_4)SO_4$, and 1.7 mM sodium citrate) was added to make the final volume to 1
30 ml.

E. 'bead elution'

After the final washing liquid was drawn out (section 2C), 1 ml of Min A salts solution was added to the beads. This bead mixture was added directly to a concentrated bacteria sample for infection (section 3A and 3B).

5 3. Amplification

A. Preparation of plating cells

Fresh E. Coli. (XL-1 Blue MRF') culture was grown to $OD_{600} = 0.5$ in LB media containing 12.5 µg/ml tetracycline. For each panning condition, 20 ml of this culture was chilled on ice and centrifuged. The bacteria pellet was
10 resuspended in 1 ml of the Min A Salts solution.

B. Transduction

Each mixture from different elution methods (section 2D and 2E) was added to a concentrated bacteria sample (section 3A) and incubated at 37 °C for 15 minutes. 2 ml of NZCYM media (2XNZCYM, 50 µg/ml ampicillin) was
15 added to each mixture and incubated at room temperature for 15 minutes. The resulting 4 ml solution was plated on a large NZCYM agar plate containing 50 µg/ml ampicillin and incubated overnight at 37 °C.

C. Phage Harvesting

Each of the bacteria/phage mixture that was grown overnight on a large
20 NZCYM agar plate (section 3B) was scraped off in 35 ml of LB media, and the agar plate was further rinsed with additional 35 ml of LB media. The resulting bacteria/phage mixture in LB media was centrifuged to pellet the bacteria away. 50 ml of the phage supernatant was transferred to a fresh tube, and 12.5 ml of PEG solution (20% PEG8000, 3.5M ammonium acetate) was added and incubated
25 on ice for 2 hours to precipitate phages. Precipitated phages were centrifuged down and resuspended in 6 ml of the phage resuspension buffer (250 mM NaCl, 100 mM Tris pH8, 1 mM EDTA). This phage solution was further purified by centrifuging away the remaining bacteria and precipitating the phage for the second time by adding 1.5 ml of the PEG solution. After a centrifugation step, the
30 phage pellet was resuspended in 400 µl of PBS. This solution was subjected to a final centrifugation to rid of remaining bacteria debris. The resulting phage

preparation was titered by a standard plaque formation assay (Molecular Cloning, Maniatis et al 3rd Edition).

4. Two more rounds of selection and amplification.

In the second round, the amplified phage (10^{10} pfu) from the first round (section 3C) was used as the input phage to perform the selection and amplification steps (sections 2 and 3). The amplified phage (10^{10} pfu) from the 2nd round in turn was used as the input phage to perform 3rd round of selection and amplification (sections 2 and 3). After the elution steps (sections 2D and 2E) of the 3rd round, a small fraction of the eluted phage was plated out as in the plaque formation assay (section 3C). Individual plaques were picked and placed into 96 well microtiter plates containing 100 μ l of TE buffer in each well. These master plates were incubated in a 37 °C incubator for 1 hour to allow phages to elute into the TE buffer.

5. Clonal analysis (Phage ELISA and sequencing)

The phage clones were analyzed by phage ELISA and sequencing methods. The sequences were ranked based on the combined results from these two assays.

A. Phage ELISA

An XL-1 Blue MRF' culture was grown until OD₆₀₀ reaches 0.5. 30 μ l of this culture was aliquoted into each well of a 96 well microtiter plate. 10 μ l of eluted phage (section 4) was added to each well and allowed to infect bacteria for 15 min at room temperature. 130 μ l of LB media containing 12.5 μ g/ml of tetracycline and 50 μ g/ml of ampicillin was added to each well. The microtiter plate was then incubated overnight at 37 °C. The recombinant TALL-1 protein (1 μ g/ml in PBS) was allowed to coat onto the 96-well Maxisorp plates (NUNC) overnight and 4°C. As a control, the recombinant Fc-Trail protein was coated onto a separate Maxisorp plate at the same molar concentration as the TALL-1 protein.

On the following day, liquids in the protein coated Maxisorp plates were discarded, and each well was blocked with 300 μ l of 2% BSA solution at 37 °C

for one hour. The BSA solution was discarded, and the wells were washed three times with the PBST solution. After the last washing step, 50 μ l of PBST was added to each well of the protein coated Maxisorp plates. Each of the 50 μ l overnight cultures in the 96 well microtiter plate was transferred to the

5 corresponding wells of the TALL-1 coated plates as well as the control Fc-Trail coated plates. The 100 μ l mixtures in the two kinds of plates were incubated for 1 hour at room temperature. The liquid was discarded from the Maxisorp plates, and the wells were washed five times with PBST. The HRP-conjugated anti-M13 antibody (Pharmacia) was diluted to 1:7,500, and 100 μ l of the diluted solution

10 was added to each well of the Maxisorp plates for 1 hour incubation at room temperature. The liquid was again discarded and the wells were washed seven times with PBST. 100 μ l of tetramethylbenzidine (TMB) substrate (Sigma) was added to each well for the color reaction to develop, and the reaction was stopped with 50 μ l of the 5 N H₂SO₄ solution. The OD₄₅₀ was read on a plate

15 reader (Molecular Devices).

B. Sequencing of the phage clones.

For each phage clone, the sequencing template was prepared by a PCR method. The following oligonucleotide pair was used to amplify about 500 nucleotide fragment:

20 primer #1 (5'-CGGCGCAACTATCGGTATCAAGCTG-3') (SEQ ID NO: 56)
and primer #2 (5'-CATGTACCGTAACACTGAGTTTCGTC-3'). (SEQ ID NO: 57)

The following mixture was prepared for each clone.

Reagents	volume (μ L) / tube
dH ₂ O	26.25
50% glycerol	10
10B PCR Buffer (w/o MgCl ₂)	5
25 mM MgCl ₂	4
10 mM dNTP mix	1
100 μ M primer 1	0.25
100 μ M primer 2	0.25
Taq polymerase	0.25
Phage in TE (section 4)	3
Final reaction volume	50

The thermocycler (GeneAmp PCR System 9700, Applied Biosystems) was used to run the following program: 94°C for 5 min; [94°C for 30 sec, 55°C for 30 sec, 72°C for 45 sec.]x30 cycles; 72°C for 7 min; cool to 4°C. The PCR product was checked by running 5 µl of each PCR reaction on a 1% agarose gel. The PCR product in the remaining 45 µl from each reaction was cleaned up using the QIAquick Multiwell PCR Purification kit (Qiagen), following the manufacturer's protocol. The resulting product was then sequenced using the ABI 377 Sequencer (Perkin-Elmer) following the manufacturer recommended protocol.

6. Sequence ranking and consensus sequence determination

A. Sequence ranking

The peptide sequences that were translated from variable nucleotide sequences (section 5B) were correlated to ELISA data. The clones that showed high OD₄₅₀ in the TALL-1 coated wells and low OD₄₅₀ in the Fc-Trail coated wells were considered more important. The sequences that occur multiple times were also considered important. Candidate sequences were chosen based on these criteria for further analysis as peptides or peptibodies. Five and nine candidate peptide sequences were selected from the TN8-IX and TN12-I libraries, respectively.

B. Consensus sequence determination

The majority of sequences selected from the TN12-I library contained a very conserved DBL motif. This motif was also observed in sequences selected from the TN8-IB library as well. Another motif, PFPWE (SEQ ID NO: 110) was also observed in sequences obtained from the TN8-IB library.

A consensus peptide, FHDCKWDLLTKQWVCHGL (SEQ ID NO: 58), was designed based on the DBL motif. Since peptides derived from the TN12-I library were the most active ones, the top 26 peptide sequences based on the above ranking criteria (section 5A) were aligned by the DBL motif. The underlined "core amino acid sequence" was obtained by determining the amino acid that occur the most in each position. The two cysteines adjacent to the core

sequences were fixed amino acids in the TN12-I library. The rest of the amino acid sequence in the consensus peptide is taken from one of the candidate peptides, TALL-1-12-10 (Table 2, SEQ ID NO: 37). The peptide and peptibody that was derived from this consensus sequence were most active in the B cell proliferation assay.

EXAMPLE 2

Peptibodies

A set of 12 TALL-1 inhibitory peptibodies (Table 5) was constructed in which a monomer of each peptide was fused in-frame to the Fc region of human IgG1. Each TALL-1 inhibitory peptibody was constructed by annealing the pairs of oligonucleotides shown in Table 6 to generate a duplex encoding the peptide and a linker comprised of 5 glycine residues and one valine residue as an NdeI to SalI fragment. These duplex molecules were ligated into a vector (pAMG21-RANK-Fc, described herein) containing the human Fc gene, also digested with NdeI and SalI. The resulting ligation mixtures were transformed by electroporation into *E. coli* strain 2596 cells (GM221, described herein). Clones were screened for the ability to produce the recombinant protein product and to possess the gene fusion having the correct nucleotide sequence. A single such clone was selected for each of the peptibodies. The nucleotide and amino acid sequences of the fusion proteins are shown in Figure 4A through 4F.

Table 5. Peptide sequences and oligonucleotides used to generate TALL-1 inhibitory peptibodies.

Peptibody	Peptibody SEQ ID NO	Peptide Sequence	Sense oligo- nucleotide	Antisense oligo- nucleotide
TALL-1-8-1-a	29	PGTCFPPFWECTHA	2517-24	2517-25
TALL-1-8-2-a	30	WGACWPPFWECFKE	2517-26	2517-27
TALL-1-8-4-a	31	VPFCDLLTKHCFEA	2517-28	2517-29
TALL-1-12-4-a	32	GSRCKYKWDVLTKQCFHH	2517-30	2517-31
TALL-1-12-3-a	33	LPGCKWDLLIKQWVCDPL	2517-32	2517-33
TALL-1-12-5-a	34	SADCFDILTKSDVCTSS	2517-34	2517-35
TALL-1-12-8-a	35	SDDCMYDQLTRMFICSNL	2517-36	2517-37
TALL-1-12-9-a	36	DLNCKYDELTYKEWCQFN	2521-92	2521-93

TALL-1-12-10-a	37	FHDCKYDLLTRQM VCHGL	2521-94	2521-95
TALL-1-12-11-a	38	RNHCFWDHLLKQD ICPS	2521-96	2521-97
TALL-1-12-14-a	39	ANQCWWSLTKKNVCEFF	2521-98	2521-99
TALL-1-consensus	58	FHDCKWDL LTKQW VCHGL	2551-48	2551-49

Table 5B TALL-1 inhibitory peptibodies.

Peptibody	Peptibody SEQ ID NO	Peptide Sequence			
TALL-1-8-1-a	111	MPGTCFPPFW	ECTHAGGGGG	VDKTHTCPPC	PAPELLGGPS
		VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV
		DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY
		KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDEL T
		KNQVSLTCLV	KGFYP SDIAV	EWESNGQPEN	NYKTT PPVLD
		SDGSFFLYSK	LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK
		SLSLSPGK			
TALL-1-8-2-a	112	MWGACWPPFW	ECFKEGGGGG	VDKTHTCPPC	PAPELLGGPS
		VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV
		DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY
		KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDEL T
		KNQVSLTCLV	KGFYP SDIAV	EWESNGQPEN	NYKTT PPVLD
		SDGSFFLYSK	LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK
		SLSLSPGK			
TALL-1-8-4-a	113	MVPFCDLLTK	HCFEAGGGGG	VDKTHTCPPC	PAPELLGGPS
		VFLFPPKPKD	TLMISRTPEV	TCVVVDVSHE	DPEVKFNWYV
		DGVEVHNAKT	KPREEQYNST	YRVVSVLTVL	HQDWLNGKEY
		KCKVSNKALP	APIEKTISKA	KGQPREPQVY	TLPPSRDEL T
		KNQVSLTCLV	KGFYP SDIAV	EWESNGQPEN	NYKTT PPVLD
		SDGSFFLYSK	LTVDKSRWQQ	GNVFSCSVMH	EALHNHYTQK
		SLSLSPGK			
TALL-1-12-4-a	114	MGSRCYKWD	VLTKQCFHHG	GGGGVDKTHT	CPPCPAPELL
		GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF
		NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN
		GKEYKCKVSN	KALPAPIEKT	ISKAKGQPRE	PQVYTLPPSR
		DELTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTT P
		PVLDSGDSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH
		YTQKSLSLSP	GK		
TALL-1-12-3-a	115	MLPGCKWDL	IKQWVCDPLG	GGGGVDKTHT	CPPCPAPELL
		GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF
		NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN
		GKEYKCKVSN	KALPAPIEKT	ISKAKGQPRE	PQVYTLPPSR
		DELTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTT P
		PVLDSGDSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH
		YTQKSLSLSP	GK		
TALL-1-12-5-a	116	MSADCYFDIL	TKSDVCTSSG	GGGG VDKTHT	CPPCPAPELL
		GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF
		NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN
		GKEYKCKVSN	KALPAPIEKT	ISKAKGQPRE	PQVYTLPPSR
		DELTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTT P
		PVLDSGDSFF	LYSKLTVDKS	RWQQGNVFSC	SVMHEALHNH
		YTQKSLSLSP	GK		
TALL-1-12-8-a	117	MSDDCMYDQL	TRMFICSNLG	GGGGVDKTHT	CPPCPAPELL
		GGPSVFLFPP	KPKDTLMISR	TPEVTCVVVD	VSHEDPEVKF
		NWYVDGVEVH	NAKTKPREEQ	YNSTYRVVSV	LTVLHQDWLN
		GKEYKCKVSN	KALPAPIEKT	ISKAKGQPRE	PQVYTLPPSR
		DELTKNQVSL	TCLVKGFYPS	DIAVEWESNG	QPENNYKTT P

		PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1-12-9-a	118	MDLNCKYDEL TYKEWCQFNG GGGGVDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1-12-10-a	119	MFHDCKYDLL TRQMVCHGLG GGGGVDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1-12-11-a	120	MRNHCFWDHL LKQDICPSPG GGGGVDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1-12-14-a	121	MANQCWWDLS TKKNVCEFFG GGGGVDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1-consensus	122	MFHDCKWDLL TKQWVCHGLG GGGGVDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR DELTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTT PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNN YTQKSLSLSP GK
TALL-1 12-3 tandem dimer	123	MLPGCKWDLL IKQWVCDPLG SGSATGGSGS TASSGSGSAT HMLPGCKWDL LIKQWVCDPL GGGGVDKTH TCPPCPAPEL LGGPSVFLFP KPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPRE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLSP PGK
TALL-1 consensus tandem dimer	124	MFHDCKWDLL TKQWVCHGLG SGSATGGSGS TASSGSGSAT HMFHDCKWDL LTKQWVCHGL GGGGVDKTH TCPPCPAPEL LGGPSVFLFP KPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPRE QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS RDELTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSGDSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLSP PGK

Table 6. Sequences of oligonucleotides used in peptibody construction.

Oligo-nucleotide ID number	SEQ ID NO	Sequence
2517-24	71	TAT GCC GGG TAC TTG TTT CCC GTT CCC GTG GGA ATG CAC TCA CGC TGG TGG AGG CGG TGG GG
2517-25	72	TCG ACC CCA CCG CCT CCT GGA GCG TGA GTG CAT TCC CAC GGG AAG CCG AAA CAA GTA CCC GGC A
2517-26	73	TAT GTG GGG TGC TTG TTG GCC GTT CCC GTG GGA ATG TTT CAA AGA AGG TGG AGG CGG TGG GG
2517-27	74	TCG ACC CCA CCG CCT CCA CCT TCT TTG AAA CAT TCC CACGGG AAC GGC CAA CAAGCA CCC CAC A
2517-28	75	TAT GGT TCC GTT CTG TGA CCT GCT GAC TAA ACA CTG TTT CGA AGC TGG TGG AGG CGG TGG GG
2517-29	76	TCG ACC CCA CCG CCT CCA CCA GCT TCG AAA CAG TGT TTA GTC AGC AGG TCA CAGAAC GGA ACC A
2517-30	77	TAT GGG TTC TCG TTG TAA ATA CAA ATG GGA CGT TCT GAC TAA ACA GTG TTT CCA CCA CGG TGG AGG CGG TGG GG
2517-31	78	TCG ACC CCA CCG CCT CCA CCG TGG TGG AAA CAC TGT TTA GTC AGA ACG TCC CAT TTG TAT TTA CAA CGA GAA CCC A
2517-32	79	TAT GCT GCC GGG TTG TAA ATG GGA CCT GCT GAT CAA ACA GTG GGT TTG TGA CCC GCT GGG TGG AGG CGG TGG GG
2517-33	80	TCG ACC CCA CCG CCT CCA CCC AGC GGG TCA CAA ACC CAC TGT TTG ATC AGC AGG TCC CAT TTA CAA CCC GGC AGC A
2517-34	81	TAT GTC TGC TGA CTG TTA CTT CGA CAT CCT GAC TAA ATC TGA CGT TTG TAC TTC TTC TGG TGG AGG CGG TGG GG
2517-35	82	TCG ACC CCA CCG CCT CCA CCA GAA GAA GTA CAA ACG TCA GAT TTA GTC AGG ATG TCG AAG TAA CAG TCA GCA GAC A
2517-36	83	TAT GTC TGA CGA CTG TAT GTA CGA CCA GCT GAC TCG TAT GTT CAT CTG TTC TAA CCT GGG TGG AGG CGG TGG GG
2517-37	84	TCG ACC CCA CCG CCT CCA CCC AGG TTA GAA CAG ATG AAC ATA CGA GTC AGC TGG TCG TAC ATA CAG TCG TCA GAC A
2521-92	85	TAT GGA CCT GAA CTG TAA ATA CGA CGA ACT GAC TTA CAA AGA ATG GTG TCA GTT CAA CGG TGG AGG CGG TGG GG
25221-93	86	TCG ACC CCA CCG CCT CCA CCG TTG AAC TGA CAC CAT TCT TTG TAA GTC AGTTCG TCG TAT TTA CAG TTC AGG TCC A
2521-94	87	TAT GTT CCA CGA CTG TAA ATA CGA CCT GCT GAC TCG TCA GAT GGT TTG TCA CGG TCT GGG TGG AGG CGG TGG GG
2521-95	88	TCG ACC CCA CCG CCT CCA CCC AGA CCG TGA CAA ACC ATC TGA CGA GTC AGC AGG TCG TAT TTA CAG TCG TGG AAC A
2521-96	89	TAT GCG TAA CCA CTG TTT CTG GGA CCA CCT GCT GAA ACA

		GGA CAT CTG TCC GTC TCC GGG TGG AGG CGG TGG GG
2521-97	90	TCG ACC CCA CCG CCT CCA CCC GGA GAC GGA CAG ATG TCC TGT TTC AGC AGG TGG TCC CAG AAA CAG TGG TTA CGC A
2521-98	91	TAT GGC TAA CCA GTG TTG GTG GGA CTC TCT GCT GAA AAA AAA CGT TTG TGA ATT CTT CGG TGG AGG CGG TGG GG
2521-99	92	TCG ACC CCA CCG CCT CCA CCG AAG AAT TCA CAA ACG TTT TTT TTC AGC AGA GAG TCC CAC CAA CAC TGG TTA GCC A
2551-48	93	TAT GTT CCA CGA CTG CAA ATG GGA CCT GCT GAC CAA ACA GTG GGT TTG CCA CGG TCT GGG TGG AGG CGG TGG GG
2551-49	94	TCG ACC CCA CCG CCT CCA CCC AGA CCG TGG CAA ACC CAC TGT TTG GTC AGC AGG TCC CAT TTG CAG TCG TGG AAC A

pAMG21-RANK-Fc vector

pAMG21. The expression plasmid pAMG21 (ATCC accession no. 98113) can be derived from the Amgen expression vector pCFM1656 (ATCC #69576) which in turn be derived from the Amgen expression vector system described in US Patent No. 4,710,473. The pCFM1656 plasmid can be derived from the described pCFM836 plasmid (U.S. Patent No. 4,710,473) by:

- destroying the two endogenous NdeI restriction sites by end filling with T4 polymerase enzyme followed by blunt end ligation;
- replacing the DNA sequence between the unique AatII and ClaI restriction sites containing the synthetic P_L promoter with a similar fragment obtained from pCFM636 (patent No. 4,710,473) containing the P_L promoter (see SEQ ID NO: 95 below); and
- substituting the small DNA sequence between the unique ClaI and KpnI restriction sites with the oligonucleotide having the sequence of SEQ ID NO: 96.

SEQ ID NO: 95:

AatII
 5' CTAATTCGGCTCTCACCTACCAAACAATGCCCCCTGCAAAAAATAAATTCATAT-
 20 3' TGCAGATTAAGGCGAGAGTGGATGGTTTGTACGGGGGACGTTTTTTATTTAAGTATA-
 -AAAAAACATACAGATAACCATCTGCGGTGATAAATTATCTCTGGCGGTGTTGACATAAA-
 -TTTTTGTATGTCTATTGGTAGACGCCACTATTTAATAGAGACCGCCACAACGTGATTT-
 25 -TACCACTGGCGGTGATACTGAGCACAT 3'
 -ATGGTGACCGCCACTATGACTCGTGTAGC 5'
ClaI

SEQ ID NO: 96:

5' CGATTTGATTCTAGAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGGTAC
 3' TAAACTAAGATCTTCTCCTTATTGTATACCAATTGCGCAACCTTAAGC 5'
ClaI KpnI

5 The expression plasmid pAMG21 can then be derived from pCFM1656 by making a series of site-directed base changes by PCR overlapping oligonucleotide mutagenesis and DNA sequence substitutions. Starting with the BglII site (plasmid bp # 180) immediately 5' to the plasmid replication promoter P_{copB} and
 10 proceeding toward the plasmid replication genes, the base pair changes are as shown in Table 7 below.

Table 7—Base pair changes resulting in pAMG21

	<u>pAMG21 bp #</u>	<u>bp in pCFM1656</u>	<u>bp changed to in pAMG21</u>
15	# 204	T/A	C/G
	# 428	A/T	G/C
	# 509	G/C	A/T
	# 617	- -	insert two G/C bp
20	# 679	G/C	T/A
	# 980	T/A	C/G
	# 994	G/C	A/T
	# 1004	A/T	C/G
	# 1007	C/G	T/A
25	# 1028	A/T	T/A
	# 1047	C/G	T/A
	# 1178	G/C	T/A
	# 1466	G/C	T/A
	# 2028	G/C	bp deletion
30	# 2187	C/G	T/A
	# 2480	A/T	T/A
	# 2499-2502	<u>AGTG</u> <u>TCAC</u>	<u>GTCA</u> <u>CAGT</u>
35	# 2642	<u>TCCGAGC</u> <u>AGGCTCG</u>	7 bp deletion
	# 3435	G/C	A/T
40	# 3446	G/C	A/T
	# 3643	A/T	T/A

The DNA sequence between the unique AatII (position #4364 in pCFM1656) and SacII (position #4585 in pCFM1656) restriction sites is
 45 substituted with the DNA sequence below (SEQ ID NO: 97):.

[AatII sticky end] 5' GCGTAACGTATGCATGGTCTCC-
(position #4358 in pAMG21) 3' TGCACGCATTGCATACGTACCAGAGG-

5 -CCATGCGAGAGTAGGGAAC TGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT-
-GGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAGCTTTCTGA-

-GGGCTTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCCTGAGTAGGACAAATCCGC-
-CCCGGAAAGCAAAATAGACAACAACAGCCACTTGCGAGAGGACTCATCCTGTTTAGGCG-

10 -CGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGGAGGGTGGCGGGCAGGACGCCCGC-
-GCCCTCGCCTAAACTTGCAACGCTTCGTTGCCGGGCCTCCACCGCCCGTCTGCGGGCG-

-CATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTGCGT-
-GTATTTGACGGTCCGTAGTTTAATTCGCTTCGCGGTAGGACTGCCTACCGGAAAAACGCA-

15 AatII
-TTCTACAACTCTTTTGTTTATTTTCTAAATACATTCAAATATGGACGTCGTACTTAAC-
-AAGATGTTTGAGAAAACAAATAAAAAGATTATGTAAGTTTATACCTGCAGCATGAATTG-

20 -TTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAATGCTTTAGAAATACTTTGGCAGC-
-AAAATTCATACCCGTTAGTTAACGAGGACAATTTAACGAAATCTTTATGAAACCGTCG-

-GGTTTGTGTATTGAGTTTCATTTGCGCATTGGTTAAATGGAAAGTGACCGTGCGCTTAC-
-CCAAACAACATAACTCAAAGTAAACGCGTAACCAATTTACCTTTCACTGGCAGCGCAATG-

25 -TACAGCCTAATATTTTTGAAATATCCCAAGAGCTTTTTCTTCGCATGCCCACGCTAAAC-
-ATGTCGGATTATAAAAACTTTATAGGGTCTCGAAAAAGGAAGCGTACGGGTGCGATTG-

-ATTCTTTTCTCTTTTGTTAAATCGTTGTTGATTATTATTGCTATATTTATTTTTC-
30 -TAAGAAAAAGAGAAAACCAATTTAGCAACAACTAAATAATAAACGATATAAATAAAAG-

-GATAATTATCAACTAGAGAAGGAACAATTAATGGTATGTTTCATACACGCATGTAAAAATA-
-CTATTAATAGTTGATCTCTTCCTTGTTAATTACCATACAAGTATGTGCGTACATTTTAT-

35 -AACTATCTATATAGTTGTCTTTCTCTGAATGTGCAAAACTAAGCATTCCGAAGCCATTAT-
-TTGATAGATATATCAACAGAAAGAGACTTACACGTTTTGATTTCGTAAGGCTTCGGTAATA-

-TAGCAGTATGAATAGGGAACTAAACCCAGTGATAAGACCTGATGATTTGCTTCTTTAA-
40 -ATCGTCATACTTATCCCTTTGATTTGGGTCACTATTCTGGACTACTAAAGCGAAGAAATT-

-TTACATTGGAGATTTTTTATTTACAGCATTGTTTTCAAATATATTCCAATTAATCGGTG-
-AATGTAAACCTCTAAAAATAAATGTGCGTAACAAAAGTTTATATAAGGTTAATTAGCCAC-

45 -AATGATTGGAGTTAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCAT-
-TTACTAACCTCAATCTTATTAGATGATATCCTAGTATAAAATAATTTAATCGCAGTAGTA-

-AATATTGCCTCCATTTTTTAGGGTAATTATCCAGAAATTGAAATATCAGATTTAACCATAG-
-TTATAACGGAGGTAAAAATCCCATTAATAGGTCTTAACCTTATAGTCTAAATTGGTATC-

50 -AATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTACCATTTTAGTCATATCAG-
-TTACTCCTATTTACTAGCGCTCATTATTATAAGTGTTACATGGTAAATCAGTATAGTC-

-ATAAGCATTGATTAATATCATTATTGCTTCTACAGGCTTTAATTTTATTAATTATTCTGT-
55 -TATTGCTAACTAATTATAGTAATAACGAAGATGTCCGAAATTAAAAATAATTAATAAGACA-

-AAGTGTCGTCGGCATTATGTCCTTTCATACCCATCTCTTTATCCTTACCTATTGTTGTC-
-TTCACAGCAGCCGTAAATACAGAAAGTATGGGTAGAGAAATAGGAATGGATAACAAACAG-

60 -GCAAGTTTTGCGTGTTATATATCATTAAACGTAATAGATTGACATTTGATTCTAATAA-
-CGTTCAAAACGCACAATATATAGTAATTTGCCATTATCTAACTGTAACTAAGATTATT-

-ATTGGATTTTTGTCACACTATTATATCGCTTGAAATACAATTGTTTAAACATAAGTACCTG-
-TAACCTAAAAACAGTGTGATAATATAGCGAATTTATGTTAAACAAATTGTATTTCATGGAC-

-TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTATAGTCGATTAATCGATTGATT-
 -ATCCTAGCATGTCCAAATGCGTTCCTTTACCAAACAATATCAGCTAATTAGCTAAACTAA-
 -CTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGA-
 5 -GATCTAAACAAAATTGATTAATTTCCCTTATTGTATACCAATTGCGCAACCTTAAGCT-

SacII

 -GCTCACTAGTGTGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAA-
 10 -CGAGTGATCACAGCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTCTCT-
 -GAAGAAGAAGAAGAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA-
 -CTTCTTCTTCTTCTTTCGGGCTTTCTTCGACTCAACCGACGACGGTGGCGACTCGTTAT-
 -ACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTGCTGAAAGGAGG-
 15 -TGATCGTATTGGGGGAACCCGGAGATTGCCCAGAACTCCCCAAAAACGACTTTCCTCC-

 -AACCGCTCTTCACGCTCTTCACGC 3' [SacII sticky end]
 -TTGGCGAGAAGTGCGAGAAGTG 5' (position #5904 in pAMG21)

20 During the ligation of the sticky ends of this substitution DNA sequence, the outside AatII and SacII sites are destroyed. There are unique AatII and SacII sites in the substituted DNA.

A gene encoding human RANK fused to the N-terminus of Fc was ligated into pAMG21 as an NdeI to BamHI fragment to generate Amgen Strain #4125. This
 25 construct was modified to insert a valine codon at the junction of RANK and Fc. The adjacent valine and aspartate codons create a unique SalI site. This allows for the fusion of peptides at the N-terminus of Fc3 between the unique NdeI and SalI sites. The RANK sequence is deleted upon insertion of a new NdeI-SalI fragment. The sequence of the vector is given in Figure 5A through 5M.

30 GM221 (Amgen #2596). The Amgen host strain #2596 is an E. coli K-12 strain derived from Amgen strain #393, which is a derivative of E. coli W1485, obtained from the E. coli Genetic Stock Center, Yale University, New Haven, Connecticut (CGSC strain 6159). It has been modified to contain both the temperature sensitive lambda repressor cI857s7 in the early ebg region and the
 35 lacI^Q repressor in the late ebg region (68 minutes). The presence of these two repressor genes allows the use of this host with a variety of expression systems, however both of these repressors are irrelevant to the expression from luxP_R. The untransformed host has no antibiotic resistances.

The ribosome binding site of the cI857s7 gene has been modified to
 40 include an enhanced RBS. It has been inserted into the ebg operon between

nucleotide position 1170 and 1411 as numbered in Genbank accession number M64441Gb_Ba with deletion of the intervening ebg sequence. The sequence of the insert is shown below with lower case letters representing the ebg sequences flanking the insert shown below (SEQ ID NO: 98):

5

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ttattttcgtGCGGCCGCACCATTTATCACCGCCAGAGGTAAACTAGTCAACACGCACGGTGTAGATAT
TTATCCCTTGCGGTGATAGATTGAGCACATCGATTTGATTCTAGAAGGAGGGATAATATATGAG
CACAAAAAAGAAACCATTAAACACAAGAGCAGCTTGAGGACGCACGTCGCCTTAAAGCAATTTA
TGAAAAAAGAAAAATGAACTTGGCTTATCCCAGGAATCTGTCGCAGACAAGATGGGGATGGG
10 GCAGTCAGGCGTTGGTGCCTTATTTAATGGCATCAATGCATTAAATGCTTATAACGCCGCATTGC
TTACAAAAATTCTCAAAGTTAGCGTTGAAGAATTTAGCCCTTCAATCGCCAGAGAATCTACGAG
ATGTATGAAGCGGTTAGTATGCAGCCGTCACCTAGAAGTGAGTATGAGTACCCTGTTTTTCTCA
TGTTACGGCAGGGATGTTCTCACCTAAGCTTAGAACCTTTACCAAAGGTGATGCGGAGAGATGG
GTAAGCACAAACCAAAAAAGCCAGTGATTCTGCATTCTGGCTTGAGGTTGAAGGTAATTCCATGA
15 CCGCACCAACAGGCTCCAAGCCAAGCTTTCCTGACGGAATGTTAATTCTCGTTGACCTGAGCA
GGCTGTTGAGCCAGGTGATTCTGCATAGCCAGACTTGGGGGTGATGAGTTTACCTTCAAGAAA
CTGATCAGGGATAGCGGTCAGGTGTTTTACAACCACTAAACCCACAGTACCCAATGATCCCAT
GCAATGAGAGTTGTTCCGTTGTGGGGAAGTTATCGCTAGTCAGTGGCCTGAAGAGACGTTTGG
20 CTGATAGACTAGTGGATCCACTAGTgtttctgcc

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20

The construct was delivered to the chromosome using a recombinant phage called MMeBg-cI857s7enhanced RBS #4 into F'tet/393. After recombination and resolution only the chromosomal insert described above remains in the cell. It was renamed F'tet/GM101. F'tet/GM101 was then modified

25 by the delivery of a lacI^Q construct into the ebg operon between nucleotide position 2493 and 2937 as numbered in the Genbank accession number M64441Gb_Ba with the deletion of the intervening ebg sequence. The sequence of the insert is shown below with the lower case letters representing the ebg sequences flanking the insert (SEQ ID NO: 99) shown below:

30

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ggcggaaaccGACGTCCATCGAATGGTGCAAAACCTTTTCGCGGTATGGCATGATAGCGCCCGGAAGA
GAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGT
GTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAAACGCGGG
35 AAAAAAGTCGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACCTGG
CGGGCAAACAGTCGCTCCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCGCTCGCA
AATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCTGATGGTA
GAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTG
GGCTGATCATTAATACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAA
40 TGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGA
AGACGGTACGCGACTGGGCGTGGAGCATCTGGTTCGATTGGGTACACGCAAATCGCGCTGTTA
GCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCG
CAATCAAATTCAGCCGATAGCGGAACGGGAAGCGCACTGGAGTGCCATGTCCGTTTCAACAA
45 ACCATGCAAAATGCTGAATGAGGGCATCGTTCCTCCACTGCGATGCTGGTTGCCAAGCATCAGATGG
CGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGGGATATCTCGGTAGT
GGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTTAACCAACATCAAACAGGAT
TTTCGCTGCTGGGGCAAACAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGA

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45

AGGGCAATCAGCTGTTGCCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCA
 AACCGCCTCTCCCCGCGCGTTGGCCGATTCAATTAATGCAGCTGGCAGACAGGTTTCCCGACTGG
 AAAGCGGACAGTAAGGTACCATAGGATCCaggcacagga

5 The construct was delivered to the chromosome using a recombinant
 phage called AGebg-LacIQ#5 into F'tet/GM101. After recombination and
 resolution only the chromosomal insert described above remains in the cell. It
 was renamed F'tet/GM221. The F'tet episome was cured from the strain using
 acridine orange at a concentration of 25 µg/ml in LB. The cured strain was
 10 identified as tetracycline sensitive and was stored as GM221.

Expression in E. coli. Cultures of each of the pAMG21-Fc-fusion
 constructs in E. coli GM221 were grown at 37 °C in Luria Broth medium.
 Induction of gene product expression from the luxPR promoter was achieved
 15 following the addition of the synthetic autoinducer N-(3-oxohexanoyl)-DL-
 homoserine lactone to the culture media to a final concentration of 20 ng/ml.
 Cultures were incubated at 37 °C for a further 3 hours. After 3 hours, the bacterial
 cultures were examined by microscopy for the presence of inclusion bodies and
 were then collected by centrifugation. Refractile inclusion bodies were observed
 20 in induced cultures indicating that the Fc-fusions were most likely produced in the
 insoluble fraction in E. coli. Cell pellets were lysed directly by resuspension in
 Laemmli sample buffer containing 10% β-mercaptoethanol and were analyzed by
 SDS-PAGE. In each case, an intense Coomassie-stained band of the appropriate
 molecular weight was observed on an SDS-PAGE gel.

25

EXAMPLE 3

TALL-1 peptibody inhibits TALL-1 mediated B cell proliferation

Mouse B lymphocytes were isolated from C57BL/6 spleens by negative
 selection. (MACS CD43 (Ly-48) Microbeads, Miltenyi Biotech, Auburn, CA).
 30 Purified (10^5) B cells were cultured in MEM, 10% heat inactivated FCS, 5×10^{-5} M
 2-mercaptoethanol, 100 U/ml penicillin, 100 µg/ml streptomycin) in triplicate in
 96-well flat bottom tissue culture plates with 10 ng/ml TALL-1 protein and 2
 µg/ml of Goat F(ab')₂ anti-mouse IgM (Jackson ImmunoResearch Laboratory,

West Grove, Pennsylvania) with the indicated amount of recombinant TALL-1 peptibody for a period of 4 days at 37 °C, 5%CO₂. Proliferation was measured by the uptake of radioactive ³[H] thymidine after an 18-hour incubation period.

5

EXAMPLE 4

TALL-1 peptibody blocks TALL-1 binding to its receptors

Reacti-Gel 6x (Pierce) were pre-coated with human AGP3 (also known as TALL-1, Khare et al., Proc. Natl. Acad. Sci. 97:3370-3375, 2000) and blocked with BSA. 100 pM and 40 pM of AGP3 peptibody samples were incubated with indicated various concentrations of human AGP3 at room temperature for 8 hours before run through the human AGP3-coated beads. The amount of the bead-bound peptibody was quantified by fluorescent (Cy5) labeled goat anti-human-Fc antibody (Jackson Immuno Research). The binding signal is proportional to the concentration of free peptibody at binding equilibrium. Dissociation equilibrium constant (K_D) was obtained from nonlinear regression of the competition curves using a dual-curve one-site homogeneous binding model (KinEx™ software). K_D is about 4 pM for AGP3 peptibody (SEQ ID NO: 123) binding with human AGP3 (Figure 10).

To determine if this AGP3 peptibody can neutralize murine AGP3 binding as well as human AGP3, a BIAcore neutralizing assay was utilized. All experiments were performed on a BIAcore 3000 at room temperature. Human TACI-Fc protein (Xia et al, J. Exp. Med. 192, 137-144, 2000) was immobilized to a B1 chip using 10 mM Acetate pH 4.0 to a level of 2900RU. A blank flow cell was used as a background control. Using a running buffer of PBS (without calcium or magnesium) containing 0.005% P20, 1 nM recombinant human AGP3 (in running buffer plus, 0.1 mg/ml BSA) was incubated without and with indicated various amount of AGP3 peptibody (x axis) before injected over the surface of the receptor. Regeneration was performed using 8 mM glycine pH 1.5 for 1 minute, 25 mM 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) pH 10.5, 1 M NaCl for 1 minute. For determination of murine AGP3 binding, human his-tagged

TACI was immobilized to 1000 RU in the above buffer. 5 nM recombinant murine AGP3 (in running buffer plus, 0.1 mg/ml BSA) was incubated without and with the various amounts indicated in Figure 11 of AGP3 peptibody (x axis) before injected over the surface of the receptor. Regeneration was performed with 10 mM HCl pH2, twice for 30 seconds. Relative binding of both human and murine AGP3 at presence vs absence of AGP3 peptibody (SEQ ID NO: 123) was measured (y axis). Relative binding response was determined as (RU-RU blank/RUo-RU blank). The AGP3 peptibody (SEQ ID NO: 123) inhibited both human and murine AGP3 binding to its receptor TACI (Figures 11A and 11B).

To examine if this AGP3 peptibody blocks AGP3 binding to all three receptors (TACI, BCMA and BAFFR), recombinant soluble receptor TACI, BCMA and BAFFR proteins were immobilized to CM5 chip. Using 10 mM acetate, pH4, human TACI-Fc was immobilized to 6300 RU, human BCMA-Fc to 5000 RU, and BAFFR-Fc to 6000 RU. 1 nM of recombinant human AGP3 (in running buffer containing 0.1 mg/ml BSA and 0.1 mg/ml Heparin) or 1 nM recombinant APRIL protein (Yu, et al., Nat. Immunol., 1:252-256, 2000) were incubated with indicated amount of AGP3 peptibody before injection over each receptor surface. Regeneration for the AGP3 experiment was done with 8 mM glycine, pH 1.5, for 1 minute, followed by 25 mM CAPS, pH 10.5, 1M NaCl for 1 minute. Regeneration for the APRIL experiment was performed with 8 mM glycine, pH 2, for one minute, followed by 25 mM CAPS, pH 10.5, 1 M NaCl for one minute. Relative binding of AGP3 or APRIL was measured. AGP3 peptibody (SEQ ID NO: 123) blocked AGP3 binding to all three receptors (Figure 12A). AGP3 peptibody didn't affect APRIL binding to the receptors (Figure 12B).

25

EXAMPLE 5

AGP3 peptibody blocks AGP3 mediated B cell proliferation

Mouse B lymphocytes were isolated from C57BL/6 spleens by negative selection. (MACS CD43 (Ly-48) Microbeads, Miltenyi Biotech, Auburn, CA).

30

Purified (10^5) B cells were cultured in minimal essential medium (MEM), 10% heat inactivated fetal calf serum (FCS), 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 μ g/ml streptomycin) in triplicate in 96-well flat bottom tissue culture plates with 10 ng/ml AGP3 (TALL-1) protein and 2 μ g/ml of Goat F(ab')₂ anti-mouse IgM (Jackson ImmunoResearch Laboratory, West Grove, Pennsylvania) with the indicated amount of recombinant AGP3 peptibody (SEQ ID NO: 123) for a period of 4 days at 37 °C, 5% CO₂. Proliferation was measured by the uptake of radioactive ³[H] thymidine after an 18-hour incubation period.

10

EXAMPLE 6

AGP3 peptibody on AGP3-stimulated Ig production in mice

Mice (Balb/c females of 9-14 weeks of age and 19-21 g of weight) were purchased from Charles River Laboratories, Wilmington, MA. Mice (n = 10) were treated i.p. with 1 mg/Kg of human AGP3 once a day for five consecutive days followed by 5 mg/Kg or 0.5 mg/Kg of AGP3 peptibody (SEQ ID NO: 123) or by saline or by 5 mg/Kg of human Fc. Other mice were left untreated. Mice were sacrificed on the sixth day to measure serum IgM and IgA, which were measured by ELISA. Briefly, plates were coated with capture antibodies specific for IgM or IgA (Southern Biotechnology Associates, Birmingham, AL), blocked, and added with dilutions of standard (IgM from Calbiochem, San Diego, CA and IgA from Southern Biotechnology Associates) or test samples. Captured Ig were revealed using biotinylated antibodies specific for IgM or IgA (Southern Biotechnology Associates), neutravidin-conjugated peroxidase (Pierce, Rockford, IL), and tetramethylbenzidine (TMB) microwell peroxidase substrate (KPL, Gaithersburg, MD). Optical densities were quantitated in a Thermomax ELISA reader (Molecular Devices, Menlo Park, CA).

Human AGP3-stimulated increase in serum levels of IgM and IgA was blocked by 5 mg/Kg of the anti-AGP3 peptibody (SEQ ID NO: 123) and not by 0.5 mg/Kg (Figures 14A and 14B).

30

EXAMPLE 7

AGP3 peptibody reduced spleen B cell number in mice

Mice (as above, n = 7) were treated i.p. for seven consecutive days with 5
5 mg/Kg or 1.5 mg/Kg or 0.5 mg/Kg of AGP3 peptibody (SEQ ID NO: 123) or with
saline or with 5 mg/Kg of human Fc. Mice were sacrificed on the eighth day to
count spleen B cell number. Spleens were collected in saline and gently disrupted
by manual homogenization to yield a cell suspension. The total cell number was
obtained with a H1E counter (Technicon, Tarrytown, NY). Percentages of B cells
10 were derived by immunofluorescence double staining and flow cytometry using
fluorescein isothiocyanate (FITC)-conjugated and phycoerythrin (PE)-
conjugated Ab against CD3 and B220, respectively (PharMingen, San Diego,
CA) and a FACScan analyser (Becton and Dickinson, Mountain View, CA). B
cells were identified for being CD3-B220+. At all doses, the AGP3 peptibody
15 (SEQ ID NO: 123) decreased spleen B cell number in a dose-response fashion
(Figure 14) (SEQ ID NO: 123).

EXAMPLE 8

AGP3 peptibody reduced arthritis severity in mouse CIA model

20 Eight to 12 week old DBA/1 mice (obtained from Jackson Laboratories,
Bar Harbor, ME) were immunized with bovine collagen type II (bCII) (purchased
from University of Utah), emulsified in complete Freund's adjuvant (Difco)
intradermally at the base of tail. Each injection was 100 µl containing 100 µg of
25 bCII. Mice were boosted 3 weeks after the initial immunization with bCII
emulsified in incomplete Freund's adjuvant. Treatment was begun from the day of
booster immunization for 4 weeks. Mice were examined for the development of
arthritis. As described before (Khare et al., J. Immunol. 155: 3653-9, 1995), all
four paws were individually scored from 0-3. Therefore arthritis severity could
30 vary from 0 to 12 for each animal. AGP3 (SEQ ID NO: 123) peptibody treatment
significantly reduced the severity of arthritic scores (Figure 15).

Serum samples were taken one week after final treatment (day 35) for the analysis of anti-collagen antibody level. High binding ELISA plates (Immulon, Nunc) were coated with 50 μ l of 4 μ g/ml solution of bovine CII in carbonate buffer and plated were kept in cold overnight in the refrigerator. Plates were
5 washed three times with cold water. 75 μ l of blocking solution made up of PBS/.05% tween 20/1% BSA was used to block non-specific binding for an hour. Samples were diluted (in blocking buffer) in dilution plates at 1:25, 1:100, 1:400, and 1:1600 and 25 μ l of these samples were added to each well of the ELISA plate for a final dilution of 100, 400, 1600, and 6400 with a final volume of 100
10 μ l/well. After incubation at room temperature for 3 hours, plates were washed three times again. 100 μ l of secondary antibody diluted in blocking buffer (rat anti-mouse IgM, IgG2a, IgG2b, IgG1, IgG3-HRP) was added to each well and plates were incubated for at least 2 hours. Plates were washed four times. 100 μ l of TMB solution (Sigma) was added to each well and the reaction was stopped
15 using 50 μ l of 25% sulfuric acid. Plates were read using an ELISA plate reader at 450 nm. OD was compared with a standard pool representing units/ml. AGP3 peptibody (SEQ ID NO: 123) treatment reduced serum anti-collagen II IgG1, IgG3, IgG2a, and IgG2b levels compared to PBS or Fc control treatment groups (Figure 16).

20

EXAMPLE 9

Treatment of AGP3 peptibody in NZB/NZW lupus mice

Five month old lupus prone NZBx NZBWF1 mice were treated i.p.
25 3X/week for 8 weeks with PBS or indicated doses of AGP3 peptibody or human Fc proteins. Prior to the treatment, animals were pre-screened for protein in the urine with Albustix reagents strips (Bayer AG). Mice having greater than 100 mg/dl of protein in the urine were not included in the study. Protein in the urine was evaluated monthly throughout the life of the experiment. AGP3 peptibody
30 (SEQ ID NO: 123) treatment led to delay of proteinuria onset and improved survival (Figure 17).

AGP3 peptibody treatment reduced B cell number in mice. Balb/c mice received 7 daily intraperitoneal injections of indicated amount of AGP3 peptibody (SEQ ID NO: 123) or human Fc protein. On day 8, spleens were collected, and subject to FACS analysis for B220+ B cells as set for in Table 8.

5

Table 8
AGP3 Pb Reduces B Cell Number in Normal Mice

n=7	dose (1/dayx7)	spleen B cell (1x10e6)	SD	t test
saline		51.3	9.6	
Fc	5mg/Kg	45.5	7.1	
Peptibody	5mg/Kg	20.1	3.8	1.37856E-05
	1.5mg/Kg	22.6	6.9	5.10194E-05
	0.5mg/Kg	25.8	3.6	0.000111409

10

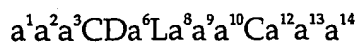
* * *

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto, without departing from the spirit and scope of the invention as set forth herein.

15

What is claimed is:

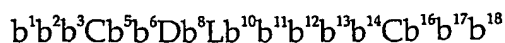
1. A TALL-1-binding composition of matter comprising an amino acid sequence Dz^2Lz^4 , wherein z^2 is an amino acid residue and z^4 is T or I,
 5 and wherein the composition of matter does not comprise a fragment of TACI, BCMA, or BAFFR (SEQ ID NOS: 195, 196, and 197).
2. The composition of matter of Claim 1, wherein z^4 is T.
3. A TALL-1-binding composition of matter comprising an amino acid sequence Dz^2LI , wherein z^2 is an amino acid residue.
- 10 4. The composition of matter of Claim 1 comprising an amino acid sequence of the formula



(SEQ. ID. NO: 100)

wherein:

- 15 a^1, a^2, a^3 are each independently absent or amino acid residues;
 a^6 is an amino acid residue;
 a^8 is T or I;
 a^9 is a basic or hydrophobic residue;
 a^{12} is a neutral polar residue; and
- 20 a^{13} and a^{14} are each independently absent or amino acid residues.
5. The composition of matter of Claim 4 wherein a^8 is T and a^9 is a basic residue.
6. The composition of matter of Claim 4 wherein a^9 is K and a^{12} is F.
7. The composition of matter of Claim 1 comprising an amino acid
 25 sequence of the formula



(SEQ. ID. NO: 104)

wherein:

- b^1 and b^2 are each independently absent or amino acid residues;
- 30 b^3 is an acidic or amide residue;

b⁵ is an amino acid residue;

b⁶ is an aromatic residue;

b⁸ is an amino acid residue;

b¹⁰ is T or I;

5 b¹¹ is a basic residue;

b¹² and b¹³ are each independently amino acid residues;

b¹⁴ is a neutral polar residue; and

b¹⁶, b¹⁷, and b¹⁸ are each independently absent or amino acid residues.

10 8. The composition of matter of Claim 7 wherein:

b³ is D, Q, or E;

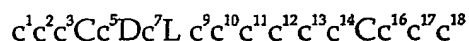
b⁶ is W or Y;

b¹⁰ is T;

b¹¹ is K or R; and

15 b¹⁴ is V or L.

9. The composition of matter of Claim 1 comprising an amino acid sequence of the formula



(SEQ. ID. NO: 105)

20 wherein:

c¹, c², and c³ are each independently absent or amino acid residues;

c⁵ is an amino acid residue;

c⁷ is an amino acid residue;

c⁹ is T or I;

25 c¹⁰ is a basic residue;

c¹¹ and c¹² are each independently amino acid residues;

c¹³ is a neutral polar residue;

c¹⁴ is an amino acid residue;

c¹⁶ is an amino acid residue;

30 c¹⁷ is a neutral polar residue; and

c^{18} is an amino acid residue or is absent.

10. The composition of matter of Claim 9 wherein:

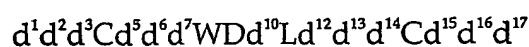
c^9 is T;

c^{10} is K or R;

5 c^{13} is a I, L, or V; and

c^{17} is A or L.

11. The composition of matter of Claim 1 comprising an amino acid sequence of the formula



10

(SEQ. ID. NO: 106)

wherein:

d^1 , d^2 , and d^3 are each independently absent or amino acid residues;

d^5 , d^6 , and d^7 are each independently amino acid residues;

d^{10} is an amino acid residue;

15

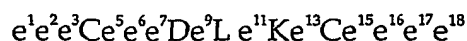
d^{13} is T or I;

d^{14} is an amino acid residue; and

d^{16} , d^{17} , and d^{18} are each independently absent or amino acid residues.

12. The composition of matter of Claim 1 comprising an amino acid sequence of the formula

20



(SEQ. ID. NO: 107)

wherein:

e^1 , e^2 , and e^3 are each independently absent or amino acid residues;

25

e^5 , e^6 , e^7 , e^9 , and e^{13} are each independently amino acid residues;

e^{11} is T or I; and

e^{15} , e^{16} , and e^{17} are each independently absent or amino acid residues.

13. The composition of matter of Claim 1 comprising an amino acid sequence of the formula



(SEQ ID NO: 109)

5 wherein:

f^1 , f^2 , and f^3 are absent or are amino acid residues;

f^5 is W, Y, or F;

f^7 is an amino acid residue;

f^9 is T or I;

10 f^{10} is K, R, or H;

f^{12} is C, a neutral polar residue, or a basic residue (W, C, or R preferred);

f^{13} is C, a neutral polar residue or is absent; and

f^{14} is any amino acid residue or is absent;

15 provided that only one of f^1 , f^2 , and f^3 may be C, and only one of f^{12} , f^{13} , and f^{14} may be C.

14. The composition of matter of Claim 13, wherein f^5 is W.

15. The composition of matter of Claim 13, wherein f^7 is L.

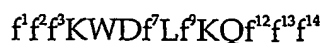
16. The composition of matter of Claim 13, wherein f^9 is T.

20 17. The composition of matter of Claim 13, wherein f^{10} is K.

18. The composition of matter of Claim 13, wherein f^{12} is C and one of f^1 , f^2 , and f^3 is C.

19. The composition of matter of Claim 13, wherein f^{13} is V.

20. The composition of matter of Claim 13 comprising an amino acid sequence of the formula



(SEQ ID NO: 125).

21. The composition of matter of Claim 20 comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 32, 33, 58,

60, 63, 66, 67, 69, 114, 115, 122, 123, 124, 147-150, 152-177, 179, 180, and 187.

22. The composition of matter of Claim 20 comprising an amino acid
5 sequence of the formula

LPGCKWDLLIKQWVCDPL (SEQ ID NO: 33).

23. A composition of matter comprising an amino acid sequence of the
formula

$$g^1 g^2 g^3 C g^5 P F g^8 W g^{10} C g^{11} g^{12} g^{13}$$

10

(SEQ. ID. NO: 101)

wherein:

g^1 , g^2 and g^3 are each independently absent or amino acid residues;

g^5 is a neutral polar residue;

g^8 is a neutral polar residue;

15

g^{10} is an acidic residue;

g^{12} and g^{13} are each independently amino acid residues; and

g^{14} is absent or is an amino acid residue.

24. The composition of matter of Claim 23 wherein:

g^2 is G;

20

g^5 is W;

g^8 is P;

g^{10} is E; and

g^{13} is a basic residue.

25. A composition of matter comprising an amino acid sequence of the
25 formula

$$h^1 h^2 h^3 C W h^6 h^7 W G h^{10} C h^{12} h^{13} h^{14}$$

(SEQ. ID. NO: 102)

wherein:

h^1 , h^2 , and h^3 are each independently absent or amino acid residues;

30

h^6 is a hydrophobic residue;

h^7 is a hydrophobic residue;

h^{10} is an acidic or polar hydrophobic residue; and

h^{12} , h^{13} , and h^{14} are each independently absent or amino acid residues.

26. The composition of matter of Claim 25 wherein:

5 h^1 is G;

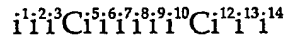
h^6 is A;

h^7 is a neutral polar residue; and

h^{10} is an acidic residue.

27. A composition of matter comprising an amino acid sequence of the

10 formula



(SEQ. ID. NO: 103)

wherein:

i^1 is absent or is an amino acid residue;

15 i^2 is a neutral polar residue;

i^3 is an amino acid residue;

i^5 , i^6 , i^7 , and i^8 are each independently amino acid residues;

i^9 is an acidic residue;

i^{10} is an amino acid residue;

20 i^{12} and i^{13} are each independently amino acid residues; and

i^{14} is a neutral polar residue.

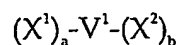
28. The composition of matter of Claim 27 wherein:

i^2 is W; and

i^{14} is W.

25 29. A TALL-1 binding composition of matter comprising an amino acid sequence of the formula PFPWE (SEQ ID NO: 110). :

30. The composition of matter of Claim 1 having the formula



and multimers thereof, wherein:

V^1 is a vehicle;

X^1 and X^2 are each independently selected from $-(L^1)_c-P^1$,

$-(L^1)_c-P^1-(L^2)_d-P^2$, $-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3$, and

$-(L^1)_c-P^1-(L^2)_d-P^2-(L^3)_e-P^3-(L^4)_f-P^4$

5 one or more of P^1 , P^2 , P^3 , and P^4 each independently comprise

Dz^2Lz^4 ;

L^1 , L^2 , L^3 , and L^4 are each independently linkers; and

a, b, c, d, e, and f are each independently 0 or 1, provided that at

least one of a and b is 1.

10 31. The composition of matter of Claim 30 of the formula

$P^1-(L^1)_c-P^2-(L^2)_d-V^1$.

32. The composition of matter of Claim 30 of the formula

$V^1-(L^1)_c-P^1-(L^2)_d-P^2$.

15 33. The composition of matter of Claim 30, wherein V^1 is an Fc domain.

34. The composition of matter of Claim 30 wherein V^1 is an IgG Fc domain.

35. The composition of matter of Claim 30 wherein V^1 is an IgG1 Fc domain.

20 36. The composition of matter of Claim 30 wherein V^1 comprises the sequence of SEQ ID NO: 2.

37. The composition of matter of Claim 30 wherein one or more of P^1 , P^2 , P^3 , and P^4 each independently comprises a sequence selected from:

$a^1a^2a^3CDa^6La^8a^9a^{10}Ca^{12}a^{13}a^{14}$ (SEQ. ID. NO: 100)

$b^1b^2b^3Cb^5b^6Db^8Lb^{10}b^{11}b^{12}b^{13}b^{14}Cb^{16}b^{17}b^{18}$ (SEQ. ID. NO: 104)

25 $c^1c^2c^3Cc^5Dc^7Lc^9c^{10}c^{11}c^{12}c^{13}c^{14}Cc^{16}c^{17}c^{18}$ (SEQ. ID. NO: 105)

$d^1d^2d^3Cd^5d^6d^7WDd^{10}Ld^{13}d^{14}d^{15}Cd^{16}d^{17}d^{18}$ (SEQ. ID. NO: 106)

$e^1e^2e^3Ce^5e^6De^9Le^{11}Ke^{13}Ce^{15}e^{16}e^{17}e^{18}$ (SEQ. ID. NO: 107)

$f^1f^2f^3Kf^5Df^7Lf^9f^{10}Qf^{12}f^{13}f^{14}$ (SEQ. ID. NO: 109)

$g^1g^2g^3Cg^5Pf^8Wg^{10}Cg^{11}g^{12}g^{13}$ (SEQ ID NO: 101),
 $h^1h^2h^3CWh^6h^7Wgh^{10}Ch^{12}h^{13}h^{14}$ (SEQ ID NO: 102), and
 $i^{1;2;3}Ci^{5;6;7;8;9;10}Ci^{12;13;14}$ (SEQ ID NO: 103)

wherein:

- 5 a^1, a^2, a^3 are each independently absent or amino acid residues;
 a^6 is an amino acid residue;
 a^9 is a basic or hydrophobic residue;
 a^8 is threonyl or isoleucyl;
 a^{12} is a neutral polar residue;
 10 a^{13} and a^{14} are each independently absent or amino acid residues;
 b^1 and b^2 are each independently absent or amino acid residues;
 b^3 is an acidic or amide residue;
 b^5 is an amino acid residue;
 b^6 is an aromatic residue;
 15 b^8 is an amino acid residue;
 b^{10} is T or I;
 b^{11} is a basic residue;
 b^{12} and b^{13} are each independently amino acid residues;
 b^{14} is a neutral polar residue;
 20 b^{16}, b^{17} , and b^{18} are each independently absent or amino acid
 residues;
 c^1, c^2 , and c^3 are each independently absent or amino acid residues;
 c^5 is an amino acid residue;
 c^7 is an amino acid residue;
 25 c^9 is T or I;
 c^{10} is a basic residue;
 c^{11} and c^{12} are each independently amino acid residues;
 c^{13} is a neutral polar residue;
 c^{14} is an amino acid residue;
 30 c^{16} is an amino acid residue;

- c^{17} is a neutral polar residue; and
 c^{18} is an amino acid residue or is absent;
 d^1, d^2 , and d^3 are each independently absent or amino acid residues;
 d^5, d^6 , and d^7 are each independently amino acid residues;
5 d^{10} is an amino acid residue;
 d^{12} is T or I;
 d^{13} is an amino acid residue; and
 d^{15}, d^{16} , and d^{17} are each independently absent or amino acid
residues;
10 e^1, e^2 , and e^3 are each independently absent or amino acid residues;
 e^5, e^6, e^7, e^9 , and e^{13} are each independently amino acid residues;
 e^{11} is T or I; and
 e^{15}, e^{16} , and e^{17} are each independently absent or amino acid residues;
 f^1, f^2 , and f^3 are absent or are amino acid residues;
15 f^6 is W, Y, or F;
 f^7 is an amino acid residue;
 f^9 is T or I;
 f^{10} is K, R, or H;
 f^{12} is C, a neutral polar residue, or a basic residue;
20 f^{13} is C, a neutral polar residue or is absent; and
 f^{14} is any amino acid residue or is absent;
provided that only one of f^1, f^2 , and f^3 may be C, and only one of f^{12} ,
 f^{13} , and f^{14} may be C;
 g^1, g^2 and g^3 are each independently absent or amino acid residues;
25 g^5 is a neutral polar residue;
 g^8 is a neutral polar residue;
 g^{10} is an acidic residue;
 g^{12} and g^{13} are each independently amino acid residues; and
 g^{14} is absent or is an amino acid residue;
30 h^1, h^2 , and h^3 are each independently absent or amino acid residues;

h^6 is a hydrophobic residue;
 h^7 is a hydrophobic residue;
 h^{10} is an acidic or polar hydrophobic residue; and
 h^{12} , h^{13} , and h^{14} are each independently absent or amino acid residues;
5 i^1 is absent or is an amino acid residue;
 i^2 is a neutral polar residue;
 i^3 is an amino acid residue;
 i^5 , i^6 , i^7 , and i^8 are each independently amino acid residues;
 i^9 is an acidic residue;
10 i^{10} is an amino acid residue;
 i^{12} and i^{13} are each independently amino acid residues; and
 i^{14} is a neutral polar residue.

38. The composition of matter of claim 37, wherein:

a^9 is a basic residue.
15 b^3 is D, Q, or E;
 b^6 is W or Y;
 b^{11} is K or R; and
 b^{14} is V or L.
 c^{10} is K or R;
20 c^{13} is a I, L, or V;
 c^{17} is A or L;
 f^5 is W;
 f^7 is L; f^7 is K; and
 f^{10} is V.

25 39. The composition of matter of Claim 37, wherein one or more of P^1 , P^2 ,
 P^3 , and P^4 each independently comprises

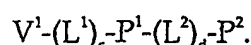
$$f^1 f^2 f^3 K W D f^7 L f^9 K Q f^{12} f^{13} f^{14}$$

(SEQ ID NO: 125).

40. The composition of matter of Claim 39 of the formula

30 $P^1-(L^1)_c-P^2-(L^2)_d-V^1$.

41. The composition of matter of Claim 39 of the formula



42. The composition of matter of Claim 39 having an amino acid sequence selected from SEQ ID NOS: 122, 123, and 124.

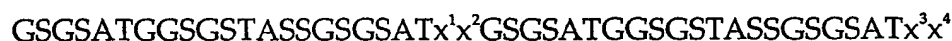
5 43. The composition of matter of Claim 40 wherein L^2 is greater than 5 amino acids.

44. The composition of matter of Claim 43 wherein L^2 is selected from



(SEQ ID NO: 193)

10 and



(SEQ ID NO: 194)

wherein x^1 and x^3 are each independently basic or hydrophobic residues and x^2 and x^4 are each independently hydrophobic residues.

15 45. The composition of matter of Claim 41 wherein L^2 is selected from



(SEQ ID NO: 59),



(SEQ ID NO: 190)

20 $\text{GSGSATGGSGSTASSGSGSATGS}$

(SEQ ID NO: 191), and



(SEQ ID NO: 192).

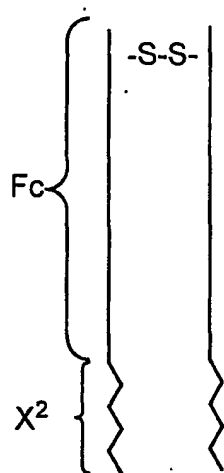
46. The composition of matter of Claim 28 comprising a sequence selected
25 from Table 2 (SEQ ID NOS: 29-39, 60-70, and 126-188).

47. The composition of matter of Claim 30 comprising a sequence selected from Table 4 (SEQ ID NOS: 44-55).

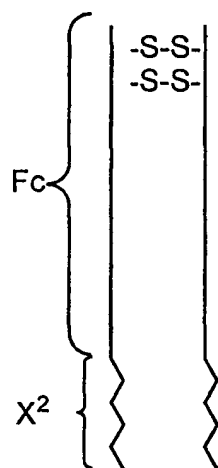
48. The composition of matter of Claim 46, wherein V^1 is an Fc domain.

49. The composition of matter of Claim 46, wherein V^1 is an IgG Fc
30 domain.

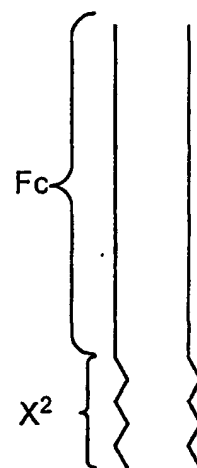
50. The composition of matter of Claim 46, wherein V¹ is an IgG1 Fc domain.
51. A DNA encoding a composition of matter of Claim 34.
52. An expression vector comprising the DNA of Claim 51.
- 5 53. A host cell comprising the expression vector of Claim 52.
54. The cell of Claim 53, wherein the cell is an E. coli cell.
55. A method of treating a B-cell mediated autoimmune disease, which comprises administering a composition of matter of Claim 1.
56. A method of treating a B-cell mediated autoimmune disease, which
10 comprises administering a composition of matter of Claim 13.
57. A method of treating lupus, which comprises administering a composition of matter of Claim 1.
58. A method of treating lupus, which comprises administering a composition of matter of Claim 13.
- 15 59. A method of treating a B-cell mediated cancer, which comprises administering a composition of matter of Claim 1.
60. A method of treating a B-cell mediated cancer, which comprises administering a composition of matter of Claim 13.
61. A method of treating B-cell lymphoma, which comprises administering
20 a composition of matter of Claim 1.
62. A method of treating B-cell lymphoma, which comprises administering a composition of matter of Claim 13.

FIG. 1

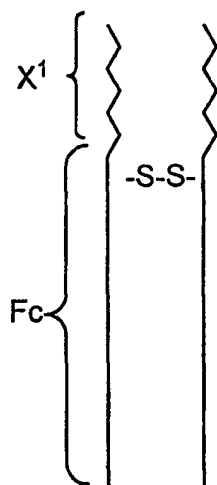
A



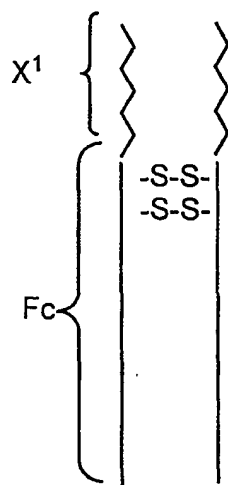
B



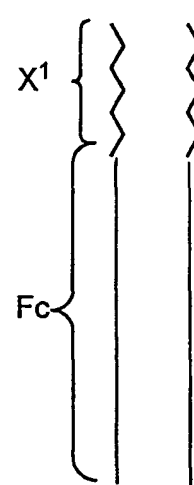
C



D



E



F

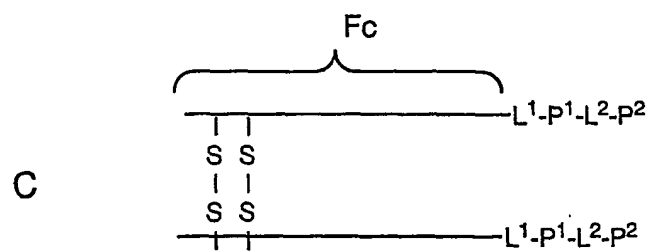
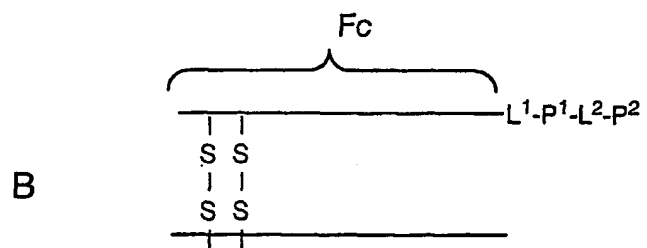
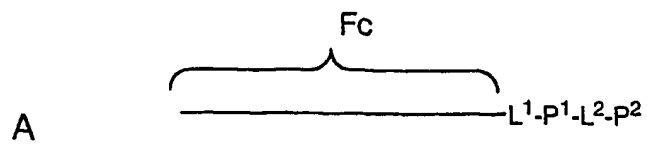
FIG. 2

FIG. 3

ATGGACAAACTCACACATGTCCACCTTGTCAGCTCCGGAACCTCTGGGGGGACCGTCA
1-----+-----+-----+-----+-----+-----+-----+ 60
TACCTGTTTTGAGTGTTACAGGTGGAACAGGTCGAGGCCTTGAGGACCCCCCTGGCAGT

a M D K T H T C P P C P A P E L L G G P S -

GTCTTCCTCTTCCCCCAAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC
61-----+-----+-----+-----+-----+-----+-----+ 120
CAGAAGGAGAAGGGGGTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGACTCCAG

a V F L F P P K P K D T L M I S R T P E V -

ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG
121-----+-----+-----+-----+-----+-----+-----+ 180
TGACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCAC

a T C V V V D V S H E D P E V K F N W Y V -

GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCAG
181-----+-----+-----+-----+-----+-----+-----+ 240
CTGCCGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCTGTC

a D G V E V H N A K T K P R E E Q Y N S T -

TACCGTGTGGTCAGCGTCCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
241-----+-----+-----+-----+-----+-----+-----+ 300
ATGGCACACCAGTCGCAGGAGTGGCAGGACGTGGTCCTGACCGACTTACCGTTCCCTCATG

a Y R V V S V L T V L H Q D W L N G K E Y -

AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCC
301-----+-----+-----+-----+-----+-----+-----+ 360
TTCACGTTCAGAGGTGTTTCGGGAGGGTCGGGGTAGCTCTTTTGGTAGAGGTTTCGG

a K C K V S N K A L P A P I E K T I S K A -

AAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGATGAGCTGACC
361-----+-----+-----+-----+-----+-----+-----+ 420
TTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGACTGG

a K G Q P R E P Q V Y T L P P S R D E L T -

AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG
421-----+-----+-----+-----+-----+-----+-----+ 480
TTCTTGGTCCAGTCGGACTGGACGGACAGTTTCCGAAGATAGGGTCGCTGTAGCGGCAC

a K N Q V S L T C L V K G F Y P S D I A V -

GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGTGGAC
481-----+-----+-----+-----+-----+-----+-----+ 540
CTCACCCCTCTCGTTACCCGTCGGCCTCTTGTGATGTTCTGGTGCGGAGGGCACGACCTG

a E W E S N G Q P E N N Y K T T P P V L D -

TCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAG
541-----+-----+-----+-----+-----+-----+-----+ 600
AGGCTGCCGAGGAAGAAGGAGATGTCGTTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTC

a S D G S F F L Y S K L T V D K S R W Q Q -

GGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG
601-----+-----+-----+-----+-----+-----+-----+ 660
CCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTC

a G N V F S C S V M H E A L H N H Y T Q K -

AGCCTCTCCCTGTCTCCGGGTAAA
661-----+-----+-----+-----+-----+-----+-----+ 684
TCGGAGAGGGACAGAGGCCCATTT

a S L S L S P G K

FIG. 4A

1) AGP3-8-1-a

NdeI

|

TATGCCGGGTACTTGTTCCTCCCGTCCCGTGGGAATGCACTCACGCTGGTGGAGGCGGT

1 -----+-----+-----+-----+-----+-----+ 60

GGCCCATGAACAAAGGGCAAGGGCACCCCTTACGTGAGTGCGACCACCTCCGCCA

a M P G T C F P F P W E C T H A G G G G -

SalI

|

GGGG

61 ----- 69

CCCCAGCT

a G V D -

2) AGP3-8-2-a

NdeI

|

TATGTGGGGTGCTTGTGGCCGTCCCGTGGGAATGTTTCAAAGAAGGTGGAGGCGGT

1 -----+-----+-----+-----+-----+-----+ 60

ACACCCACGAACAACCGGCAAGGGCACCCCTTACAAAGTTCTTCCACCTCCGCCA

a M W G A C W P F P W E C F K E G G G G -

SalI

|

GGGG

61 ----- 69

CCCCAGCT

a G V D -

FIG. 4B

3) AGP3-8-4-a

NdeI
|
TATGGTTCCGTTCTGTGACCTGCTGACTAAACACTGTTTCGAAGCTGGTGGAGGCGGT
1 -----+-----+-----+-----+-----+ 60
ACCAAGGCAAGACACTGGACGACTGATTTGTGACAAAGCTTCGACCACCTCCGCCA

a M V P F C D L L T K H C F E A G G G G -

SalI
|
GGGG
61 ----- 69
CCCCAGCT

a G V D -

4) AGP3-12-4-a

November 6, 2000 12:53 ..

NdeI
|
TATGGGTTCTCGTTGTAAATACAAATGGGACGTTCTGACTAAACAGTGTTCACCAC
1 -----+-----+-----+-----+-----+ 60
ACCCAAGAGCAACATTTATGTTTACCCTGCAAGACTGATTGTCACAAAGGTGGTG

a M G S R C K Y K W D V L T K Q C F H H -

SalI
|
GGTGGAGGCGGTGGGG
61 -----+-----+ 81
CCACCTCCGCCACCCAGCT

a G G G G G V D -

FIG. 4C

5) AGP3-12-3-a

NdeI

|

TATGCTGCCGGGTTGTAAATGGGACCTGCTGATCAAACAGTGGGTTTGTGACCCGCTG

1 -----+-----+-----+-----+-----+-----+ 60

ACGACGGCCCAACATTTACCTGGACGACTAGTTTGTACCCAAACACTGGGCGAC

a M L P G C K W D L L I K Q W V C D P L -

SalI

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT

a G G G G G V D -

6) AGP3-12-5-a

NdeI

|

TATGTCTGCTGACTGTTACTTCGACATCCTGACTAAATCTGACGTTTGTACTTCTTCT

1 -----+-----+-----+-----+-----+-----+ 60

ACAGACGACTGACAATGAAGCTGTAGGACTGATTTAGACTGCAAACATGAAGAAGA

a M S A D C Y F D I L T K S D V C T S S -

SalI

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT

a G G G G G V D -

FIG. 4D

7) AGP3-12-8-a

NdeI

|

TATGTCTGACGACTGTATGTACGACCAGCTGACTCGTATGTTTCATCTGTTCTAACCTG

1 -----+-----+-----+-----+-----+-----+-----+ 60

ACAGACTGCTGACATACATGCTGGTCGACTGAGCATAACAAGTAGACAAGATTGGAC

a M S D D C M Y D Q L T R M F I C S N L -

SalI

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT

a G G G G G V D -

8) AGP3-12-9-a

NdeI

|

TATGGACCTGAACTGTAAATACGACGAACTGACTTACAAAGAATGGTGTCAAGTTCAAC

1 -----+-----+-----+-----+-----+-----+ 60

ACCTGGACTTGACATTTATGCTGCTTGACTGAATGTTTCTTACCACAGTCAAGTTG

a M D L N C K Y D E L T Y K E W C Q F N -

SalI

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT

a G G G G G V D -

FIG. 4E

9) AGP3-12-10-a

NdeI

|

TATGTTCCACGACTGTAAATACGACCTGCTGACTCGTCAGATGGTTTGTACGGTCTG

1 -----+-----+-----+-----+-----+ 60

ACAAGGTGCTGACATTTATGCTGGACGACTGAGCAGTCTACCAAACAGTGCCAGAC

a M F H D C K Y D L L T R Q M V C H G L -

Sali

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT -

a G G G G G V D -

10) AGP3-12-11-a

NdeI

|

TATGCGTAACCACTGTTTCTGGGACCACCTGCTGAAACAGGACATCTGTCCGTCTCCG

1 -----+-----+-----+-----+-----+ 60

ACGCATTGGTGACAAAGACCCTGGTGGACGACTTTGTCCTGTAGACAGGCAGAGGC

a M R N H C F W D H L L K Q D I C P S P -

Sali

|

GGTGGAGGCGGTGGGG

61 -----+-----+-----+-----+ 81

CCACCTCCGCCACCCCAGCT

a G G G G G V D -

FIG. 4F

11) AGP3-12-14-a

|
NdeI
TATGGCTAACCAGTGTGGTGGGACTCTCTGCTGAAAAAAAAACGTTTGTGAATTCCTC
1 -----+-----+-----+-----+ 60
ACCGATTGGTCACAACCACCCTGAGAGACGACTTTTTTTTGCAAACACTTAAGAAG
a M A N Q C W W D S L L K K N V C E F F -
SalI
GGTGGAGGCGGTGGGG
61 -----+-----+ 81
CCACCTCCGCCACCCCAGCT
a G G G G G V D -

12) AGP3 Consensus

NdeI
TATGTTCCACGACTGCAAATGGGACCTGCTGACCAAACAGTGGGTTTGCCACGGTCTG
1 -----+-----+-----+-----+ 60
gtATACAAGGTGCTGACGTTTACCCTGGACGACTGGTTTGTACCCAAACGGTGCCAGAC
a M F H D C K W D L L T K Q W V C H G L -
SalI
GGTGGAGGCGGTGGGG
61 -----+-----+ 81
CCACCTCCGCCACCCCAGCT
a G G G G G V D -

P
f
l
l
l
l
0
8
I

```
GATCAGCAGTCCCCGGAACATCGTAGCTGACGCCTTCGCGTTGCTCAGTTGTCCAACCCC
1 -----+-----+-----+-----+-----+-----+-----+ 60
CTAGTCGTCAGGGGCCCTTG TAGCATCGACTGCGGAAGCGCAACGAGTCAACAGGTTGGGG

GGAAACGGGAAAAAGCAAGTTTTCCCGCTCCCGGCGTTTCAATAACTGAAAACCATACT
61 -----+-----+-----+-----+-----+-----+-----+ 120
CCTTTGCCCTTTTTCGTTCAAAGGGGCGAGGGCCGCAAAGTTATTGACTTTTGGTATGA

B
g
l
I
I
ATTTACAGTTTAAATCACATTAAACGACAGTAATCCCCGTTGATTTGTGCGCCAACACA
121 -----+-----+-----+-----+-----+-----+-----+ 180
TAAAGTGTCAAATTTAGTGTAATTTGCTGTCAATTAGGGGCAACTAAACACGCGGTTGTGT

-35 -10
----- Promoter (PcopB) ----->
GATCTTCGTCACAATTCTCAAGTCGCTGATTTCAAAAAACTGTAGTATCCTCTGCGAAAC
181 -----+-----+-----+-----+-----+-----+-----+ 240
CTAGAAGCAGTGTTAAGAGTTT CAGCGACTAAAGTTTTTTGACATCATAGGAGACGCTTTG
|--> mRNA start

GATCCCTGTTTGAGTATTGAGGAGGCGAGATGTCGCAGACAGAAAATGCAGTGACTTCCT
241 -----+-----+-----+-----+-----+-----+-----+ 300
CTAGGGACAACTCATAACTCCTCCGCTCTACAGCGTCTGTCTTTTACGTCAGTGAAGGA
M S Q T E N A V T S S -
--- copB protein --->

CATTGAGTCAAAAGCGGTTTGTGCGCAGAGGTAAGCCTATGACTGACTCTGAGAAACAAA
301 -----+-----+-----+-----+-----+-----+-----+ 360
GTAAGTCAAGTTTTCGCCAAACACGCGTCTCCATTCCGATACTGACTGAGACTCTTTGTTT
L S Q K R F V R R G K P M T D S E K Q M -

TGGCCGTTGTTGCAAGAAAACGTCTTACACACAAAAGAGATAAAAAGTTTTTGTCAAAAATC
361 -----+-----+-----+-----+-----+-----+-----+ 420
ACCGGCAACAACGTTCTTTTGCAGAATGTGTGTTTCTCTATTTTCAAAAACAGTTTTTAG
A V V A R K R L T H K E I K V F V K N P -

S
c
a
I
CTCTGAAGGATCTCATGGTTGAGTACTGCGAGAGAGAGGGGATAACACAGGCTCAGTTTCG
421 -----+-----+-----+-----+-----+-----+-----+ 480
GAGACTTCCTAGAGTACCAACTCATGACGCTCTCTCTCCCTATTGTGTCCGAGTCAAGC
L K D L M V E Y C E R E G I T Q A Q F V -
```

FIG. 5B

```

                                     -35
                                     -----
                                     ---- Promoter (PrepA) ----->
                                     |-- copB binding site --|
481 TTGAGAAAATCATCAAAGATGAACTGCAAAGACTGGATATACTAAAGTAAAGACTTTACT
-----+-----+-----+-----+-----+-----+-----+
541 AACTCTTTTAGTAGTTTCTACTTGACGTTTCTGACCTATATGATTTCATTTCTGAAATGA
c      E K I I K D E L Q R L D I L K *

                                     -10
                                     -----
541 TTGTGGCGTAGCATGCTAGATTACTGATCGTTTAAGGAATTTTGTGGCTGGCCACGCCGT
-----+-----+-----+-----+-----+-----+-----+
541 AACACCGCATCGTACGATCTAATGACTAGCAAATTCCTTAAACACCGACCGGTGCGGCA
                                     |-- mRNA -->

                                     D
                                     B r
                                     m d
                                     n I
                                     I I
                                     |<-----
601 AAGGTGGCAAGGAACCTGGTTCTGATGTGGATTTACAGGAGCCAGAAAAGCAAAAACCCCG
-----+-----+-----+-----+-----+-----+-----+
661 TTCCACCGTTCCTTGACCAAGACTACACCTAAATGTCTCGGTCTTTTTCGTTTTTGGGGC
c      M W I Y R S Q K S K N P D -
                                     --- copT (ORF) --->

<----- copA RNAI -----
661 ATAATCTTCTTCAACTTTTGCGAGTACGAAAAGATTACCGGGGCCCCACTTAAACCGTATA
-----+-----+-----+-----+-----+-----+-----+
720 TATTAGAAGAAGTTGAAAACGCTCATGCTTTTCTAATGGCCCCGGGTGAATTTGGCATAT
c      N L L Q L L R V R K D Y R G P L K P Y S -

                                     <----- Promoter (RNAI) -----
                                     -10                                     -35
                                     -----
721 GCCAACAATTCAGCTATGCGGGGAGTATAGTTATATGCCCGGAAAAGTTCAAGACTTCTTT
-----+-----+-----+-----+-----+-----+-----+
781 CGGTTGTTAAGTCGATACGCCCCCTCATATCAATATACGGGCCTTTTCAAGTTCTGAAGAA
c      Q Q F S Y A G S I V I C P E K F K T S F -

781 TCTGTGCTCGCTCCTTCTGCGCATTGTAAGTGCAGGATGGTGTGACTGATCTTACCAA
-----+-----+-----+-----+-----+-----+-----+
840 AGACACGAGCGAGGAAGACGCGTAACATTACGTCCTACCACACTGACTAGAAGTGGTTT
c      C A R S F C A L *                                     M T D L H Q T -
                                     --- repA1 protein --->

                                     D
                                     r
                                     a
                                     I
                                     I
                                     I
841 CGTATTACCGCCAGGTAAAGAACCCGAATCCGGTGTTTACACCCCGTGAAGGTGCAGGAA
-----+-----+-----+-----+-----+-----+-----+
900 GCATAATGGCGGTCCATTCTTGGGCTTAGGCCACAAATGTGGGGCACTTCCACGTCTTT
c      Y Y R Q V K N P N P V F T P R E G A G T -

901 CGCTGAAGTTCTGCGAAAACTGATGGAAAAGGCGGTGGGCTTCACTTCCCGTTTTGATT
-----+-----+-----+-----+-----+-----+-----+
960 GCGACTTCAAGACGCTTTTGTACTACCTTTTCCGCCACCCGAAGTGAAGGGCAAACTAA
c      L K F C E K L M E K A V G F T S R F D F -

```

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FIG. 5C

B
s
t
B
I

961 TCGCCATTTCATGTGGCGCACGCCCCGTTTCGCGTGATCTGCGTCGCCGTATGCCACCACTGC
-----+-----+-----+-----+-----+ 1020
AGCGGTAAGTACACCGCGTGCGGGCAAGCGCACTAGACGCAGCGGCATACGGTGGTCACG
c A I H V A H A R S R D L R R R M P P V L -

1021 TGCGTCGTCGGGCTATTGATGCGCTCTTGCAGGGGCTGTGTTTCCACTATGACCCGCTGG
-----+-----+-----+-----+-----+ 1080
ACGCAGCAGCCCGATAACTACGCGAGAACGTCCCCGACACAAAGGTGATACTGGGCGACC
c R R R A I D A L L Q G L C F H Y D P L A -

1081 CCAACCGCGTCCAGTGCTCCATCACCACGCTGGCCATTGAGTGCGGACTGGCGACGGAGT
-----+-----+-----+-----+-----+ 1140
GGTTGGCGCAGGTCACGAGGTAGTGGTGCGACCGGTAACACGCGCTGACCGCTGCCTCA
c N R V Q C S I T T L A I E C G L A T E S -

A
c
e
I
I
I

1141 CTGCTGCCGGAAAACCTCTCCATCACCCGTGCCACCCGTGCCCTGACGTTCCCTGTCAGAGC
-----+-----+-----+-----+-----+ 1200
GACGACGGCCTTTTGGAGAGGTAGTGGGCACGGTGGGCACGGGACTGCAAGGACAGTCTCG
c A A G K L S I T R A T R A L T F L S E L -

1201 TGGGACTGATTACCTACCAGACGGAATATGACCCGCTTATCGGGTGCTACATTCGGACCG
-----+-----+-----+-----+-----+ 1260
ACCCTGACTAATGGATGGTCTGCCTTATACTGGGCGAATAGCCCACGATGTAAGGCTGGC
c G L I T Y Q T E Y D P L I G C Y I P T D -

1261 ATATCACGTTTACATCTGCACTGTTTGTCTGCCCTCGATGTATCAGAGGAGGCAGTGGCCG
-----+-----+-----+-----+-----+ 1320
TATAGTGCAAGTGTAGACGTGACAAACGACGGGAGCTACATAGTCTCCTCCGTCACCGGC
c I T F T S A L F A A L D V S E E A V A A -

1321 CCGCGCGCCGCAGCCGTGTGGTATGGGAAAACAACAACGCAAAAAGCAGGGGCTGGATA
-----+-----+-----+-----+-----+ 1380
GGCGCGCGGCGTCGGCACACCATAACCTTTTGTGTTGTTGCGTTTTTCGTCCCCGACCTAT
c A R R S R V V W E N K Q R K K Q G L D T -

1381 CCCTGGGCATGGATGAAC TGATAGCGAAAGCCTGGCGTTTTGTTTCGTGAGCGTTTTTCGCA
-----+-----+-----+-----+-----+ 1440
GGGACCCGTACCTACTTGACTATCGCTTTCGGACCGCAAAACAAGCACTCGCAAAAGCGT
c L G M D E L I A K A W R F V R E R F R S -

A
f
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I
I

1441 GTTATCAGACAGAGCTTAAGTCCCCTGGAATAAAGCGTGCCCGTGCGCGTCGTGATGCGG
-----+-----+-----+-----+-----+ 1500
CAATAGTCTGTCTCGAATTCAGGGCACCTTATTTTCGCACGGGCACGCGCAGCACTACGCC
c Y Q T E L K S R G I K R A R A R R D A D -

FIG. 5D

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1501 ACAGGGAACGTCAGGATATTGTCACCCTGGTGAAACGGCAGCTGACGCGCGAAATCGCGG
-----+-----+-----+-----+-----+-----+-----+ 1560
c TGTCCCTTGCAGTCCTATAACAGTGGGACCACTTTGCCGTCGACTGCGCGCTTTAGCGCC
  R E R Q D I V T L V K R Q L T R E I A E -

1561 AAGGGCGCTTCACTGCCAATCGTGAGGCGGTAAAACGCGAAGTTGAGCGTCGTGTGAAGG
-----+-----+-----+-----+-----+-----+-----+ 1620
c TTCCCGCGAAGTGACGGTTAGCACTCCGCCATTTTGGCGTTCAACTCGCAGCACACTTCC
  G R F T A N R E A V K R E V E R R V K E -

1621 AGCGCATGATTCTGTCACGTAACCGTAATTACAGCCGGCTGGCCACAGCTTCCCCCTGAA
-----+-----+-----+-----+-----+-----+-----+ 1680
c TCGCGTACTAAGACAGTGCATTGGCATTAAATGTCGGCCGACCGGTGTCGAAGGGGGACTT
  R M I L S R N R N Y S R L A T A S . P *

1681 AGTGACCTCCTCTGAATAATCCGGCCTGCGCCGGAGGCTTCCGCACGTCTGAAGCCCGAC
-----+-----+-----+-----+-----+-----+-----+ 1740
  TCACTGGAGGAGACTTATTAGGCCGGACGCGGCCTCCGAAGGCGTCAGACTTCGGGCTG

                                     P
                                     f
                                     l
                                     M
                                     I

1741 AGCGCACAAAAAATCAGCACCACATACAAAAACAACCTCATCATCCAGCTTCTGGTGCA
-----+-----+-----+-----+-----+-----+-----+ 1800
  TCGCGTGTTTTTTTAGTCGTGGTGTATGTTTTTTGTTGGAGTAGTAGGTCGAAGACCACGT

1801 TCCGGCCCCCCTGTTTTTCGATACAAAACACGCCCTCACAGACGGGGAATTTTGCTTATCC
-----+-----+-----+-----+-----+-----+-----+ 1860
  AGGCCGGGGGGACAAAAGCTATGTTTTGTGCGGAGTGTCTGCCCCTTAAAACGAATAGG

      |----- ori -----
1861 ACATTAAACTGCAAGGGACTTCCCCATAAGGTTACAACCGTTCATGTCATAAAGCGCCAT
-----+-----+-----+-----+-----+-----+-----+ 1920
  TGTAAATTTGACGTTCCCTGAAGGGGTATTCCAATGTTGGCAAGTACAGTATTTTCGGGTA

      ----- ori -----
1921 CCGCCAGCGTTACAGGGTGCAATGTATCTTTTAAACACCTGTTTATATCTCCTTTAAACT
-----+-----+-----+-----+-----+-----+-----+ 1980
  GGCGGTCGCAATGTCCACGTTACATAGAAAATTTGTGGACAAATATAGAGGAAATTTGA

      ----- | -----
1981 ACTTAATTACATTCATTTAAAAAGAAAACCTATTCAGTGCCTGTCCTTGGACAGACAGAT
-----+-----+-----+-----+-----+-----+-----+ 2040
  TGAATTAATGTAAGTAAATTTTCTTTTGGATAAGTGACGGACAGGAACCTGTCTGTCTA

2041 ATGCACCTCCACCGCAAGCGGCGGGCCCCCTACCGGAGCCGCTTTAGTTACAACACTCAG
-----+-----+-----+-----+-----+-----+-----+ 2100
a TACGTGGAGGGTGGCGTTTCGCCGCGCGGGGATGGCCTCGGCGAAATCAATGTTGTGAGTC
  M H L P P Q A A G P Y R S R F S Y N T Q -
  --- repA4 protein ---> |----->

2101 ACACAACCACCAGAAAAACCCCGGTCCAGCGCAGAACTGAAACCACAAAGCCCCCTCCCTC
-----+-----+-----+-----+-----+-----+-----+ 2160
a TGTGTTGGTGGTCTTTTTGGGGCCAGGTCGCGTCTTGACTTTGGTGTTCGGGGAGGGAG
  T Q P P E K P R S S A E L K P Q S P S L -

2161 ATAAGTGAAGCGGCCCCCGCCCGGTCCGAAGGGCCGGAACAGAGTCGCTTTTAATTAT
-----+-----+-----+-----+-----+-----+-----+ 2220
a TATTGACTTTTCGCCGGGGCGGGCCAGGCTTCCCGGCCTTGCTCTCAGCGAAAATTAATA
  I T E K R P R P G P K G R N R V A F N Y -

```


FIG. 5E

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      GAATGTTGTAAC TACTTCATCATCGCTGTCTCAGTCTTCTCGCTGGAAGTTCTCAGTACACG
2221 -----+-----+-----+-----+-----+-----+-----+-----+ 2280
      CTTACAACATTGATGAAGTAGTAGCGACAGTCAGAAGAGCGACCTTCAAGAGTCATGTGC
a      E C C N Y F I I A V S L L A G S S Q Y T -

      BS
      gf
      li
      II
      /
      CTCGTAAGCGGCCCTGACGGCCCGCTAACGCGGAGATACGCCCCGACTTCGGGTAAACCC
2281 -----+-----+-----+-----+-----+-----+-----+ 2340
      GAGCATTTCGCCGGGACTGCCGGGCGATTGCGCCTCTATGCGGGGCTGAAGCCCATTTGGG
a      L V S G P D G P L T R R Y A P T S G K P -

      TCGTCGGGACCACTCCGACCGCGCACAGAAGCTCTCTCATGGCTGAAAGCGGGTATGGTC
2341 -----+-----+-----+-----+-----+-----+-----+ 2400
      AGCAGCCCTGGTGAGGCTGGCGCGTGTCTTCGAGAGAGTACCGACTTTTCGCCCATACCAG
a      S S G P L R P R T E A L S W L K A G M V -

      TGGCAGGGCTGGGGATGGGTAAAGGTGAAATCTATCAATCAGTACCGGCTTACGCCGGGCT
2401 -----+-----+-----+-----+-----+-----+-----+ 2460
      ACCGTCCCGACCCCTACCCATTCCACTTTAGATAGTTAGTCATGGCCGAATGCGGCCCGA
a      W Q G W G W V R *

      B
      s
      t
      E
      I
      I
      I
      TCGGCGGTTTTACTCCTGTTTCATATATGAAACAACAGGTCACCGCCTTCCATGCCGCTG
2461 -----+-----+-----+-----+-----+-----+-----+ 2520
      AGCCGCCAAAATGAGGACAAAGTATATACTTTGTTGTCCAGTGGCGGAAGGTACGGCGAC

      B
      s
      p
      L
      U
      l
      l
      I
      ATGCGGCATATCCTGGTAACGATATCTGAATTGTTATACATGTGTATATACGTGGTAATG
2521 -----+-----+-----+-----+-----+-----+-----+ 2580
      TACGCCGTATAGGACCATTGCTATAGACTTAACAATATGTACACATATATGCACCATTAC

      ACAAAAATAGGACAAGTTAAAAATTTACAGGCGATGCAATGATTCAAACACGTAATCAAT
2581 -----+-----+-----+-----+-----+-----+-----+ 2640
      TGTTTTTATCCTGTTCAATTTTTAAATGTCCGCTACGTTACTAAGTTTGTGCATTAGTTA

      ATCGGGGGTGGGCGAAGAACTCCAGCATGAGATCCCCGCGCTGGAGGATCATCCAGCCGG
2641 -----+-----+-----+-----+-----+-----+-----+ 2700
      TAGCCCCCACCCTTCTTGAGGTCGTACTCTAGGGGCGCGACCTCCTAGTAGGTCCGCC

      CGTCCCGGAAAACGATTCCGAAGCCCAACCTTTTCATAGAAGGCGGCGGTGGAATCGAAAT
2701 -----+-----+-----+-----+-----+-----+-----+ 2760
      GCAGGGCCTTTTGCTAAGGCTTCGGGTTGGAAAGTATCTCCGCCGCCACCTTAGCTTTA

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	N S P V	B l I
2761	CTCGTGATGGCAGGTTGGGCGTCGCTTGGTCGGTCATTTCGAACCCAGAGTCCCCGCTCA	2820
	-----+-----+-----+-----+-----+-----+-----+	
	GAGCACTACCGTCCAACCCGCAGCGAACCAGCCAGTAAAGCTTGGGGTCTCAGGGCGAGT	
2821	GAAGAACTCGTCAAGAAGGCCGATAGAAGGCCGATGCGCTGCGAATCGGGAGCGGCGATAACC	2880
	-----+-----+-----+-----+-----+-----+-----+	
f	* F F E D L L R Y F A I R Q S D P A A I G -	
	<--- APHII protein [kanamycin resistance gene] ---	
2881	GTAAAGCACGAGGAAGCGGTCAGCCCATTTCGCCGCCAAGCTCTTCAGCAATATCACGGGT	2940
	-----+-----+-----+-----+-----+-----+-----+	
f	CATTTCGTGCTCCTTCGCCAGTCGGGTAAAGCGGCGGTTTCGAGAAGTCGTTATAGTGCCCA	
	Y L V L F R D A W E G G L E E A I D R T -	
2941	AGCCAACGCTATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCC	3000
	-----+-----+-----+-----+-----+-----+-----+	
f	TCGGTTGCGATACAGGACTATCGCCAGGCGGTGTGGGTTCGGCCGGTGTCTAGCTACTTAGG	
	A L A I D Q Y R D A V G L R G C D I F G -	
3001	AGAAAAGCGGCCATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGAGTCACGAC	3060
	-----+-----+-----+-----+-----+-----+-----+	
f	TCTTTTCGCCGGTAAAGGTGGTACTATAAGCCGTTCTGTCGTCGTCGTCGTCGTCGTCG	
	S F R G N E V M I N P L C A D G H T V V -	
3061	GAGATCCTCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAG	3120
	-----+-----+-----+-----+-----+-----+-----+	
f	CTCTAGGAGCGGCAGCCCGTACGCGCGGAACCTCGGACCGCTTGTCAAGCCGACCGCGCTC	
	L D E G D P M R A K L R A F L E A P A L -	
3121	CCCCTGATGCTCTTCGTCCAGATCATCTGATCGACAAGACCGGCTTCCATCCGAGTACG	3180
	-----+-----+-----+-----+-----+-----+-----+	
f	GGGGACTACGAGAAGCAGGTCTAGTAGGACTAGCTGTTCTGGCCGAAGGTAGGCTCATGC	
	G Q H E E D L D D Q D V L G A E M R T R -	
3181	TGCTCGCTCGATGCGATGTTTTCGCTTGGTGGTTCGAATGGGCAGGTAGCCGGATCAAGCGT	3240
	-----+-----+-----+-----+-----+-----+-----+	
f	ACGAGCGAGCTACGCTACAAAGCGAACCACCAGCTTACCCGTCATCGGCTAGTTCGCA	
	A R E I R H K A Q H D F P C T A P D L T -	
3241	ATGCAGCCGCCGATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAAGGTGAGA	3300
	-----+-----+-----+-----+-----+-----+-----+	
f	TACGTCGGCGGGCGTAACGTAGTCGGTACTACCTATGAAAGAGCCGTCCTCGTTCCTACTCT	
	H L R R M A D A M I S V K E A P A L H S -	
3301	TGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTTCCCCTTCAGT	3360
	-----+-----+-----+-----+-----+-----+-----+	
f	ACTGTCTCTAGGACGGGGCCGTGAAGCGGGTTATCGTCGGTCAGGGAAGGGCGAAGTCA	
	S L L D Q G P V E G L L L W D R G A E T -	
3361	GACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAGCCACGATAGCCGCGC	3420
	-----+-----+-----+-----+-----+-----+-----+	
f	CTGTTGCAGCTCGTGTGACGCGTTCCTTCGCGGCGAGACCGGTCGGTGTCTATCGGCGCG	
	V V D L V A A C P V G T T A L W S L R A -	
3421	TGCCTCGTCTGCAATTCATTTCAGGACACCGGACAGGTCGGTCTTGACAAAAAGAACCGG	3480
	-----+-----+-----+-----+-----+-----+-----+	
	ACGGAGCAGGACGTTAAGTAAGTCCTGTGGCCTGTCCAGCCAGAAGTGTTCCTTCGGCC	

FIG. 5G

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f      A E D Q L E N L V G S L D T K V F L V P -
      GCGCCCCCTGCGCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCGATTGTCTGTGTGTC
3481 -----+-----+-----+-----+-----+-----+-----+ 3540
      CGCGGGGACGCGACTGTCCGGCCTTGTGCCGCCGTAGTCTCGTCCGGCTAACAGACAACACG
f      R G Q A S L R F V A A D S C G I T Q Q A -
                                     E
                                     a
                                     g
                                     I
      CCAGTCATAGCCGAATAGCCTCTCCACCCAAGCGGCCGAGAACCTGCGTGCAATCCATC
3541 -----+-----+-----+-----+-----+-----+-----+ 3600
      GGTCAGTATCGGCTTATCGGAGAGGTGGGTTCCGCCGCCCTCTTGGACGCACGTTAGGTAG
f      W D Y G F L R E V W A A P S G A H L G D -
      TTGTTCAATCATGCGAAACGATCCTCATCCTGTCTCTTGATCTGATCTTGATCCCCCTGCG
3601 -----+-----+-----+-----+-----+-----+-----+ 3660
      AACAAAGTTAGTACGCTTGCTAGGAGTAGGACAGAGAACTAGACTAGAACTAGGGGACGC
f      Q E I M
<-- APHII (kanamycin resistance) protein --)
                                     -10.
                                     <--- mRNA APHII ---|-----
3661 CCATCAGATCCTTGGCGGCAAGAAAGCCATCCAGTTTACTTTGCAGGGCTTCCCAACCTT 3720
      GGTAGTCTAGGAACCGCCGTTCTTTTCGGTAGGTCAAATGAAACGTCCCGAAGGGTTGGAA

                                     -35
                                     -----
<----- Promoter (APHII) -----
3721 ACCAGAGGGCGCCCCAGCTGGCAATTCCGGTTTCGCTTGCTGTCCATAAAACCGCCCAGTC 3780
      TGGTCTCCCGCGGGTTCGACCGTTAAGGCCAAGCGAACGACAGGTATTTTGGCGGGTCAG
      TAGCTATCGCCATGTAAGCCCACTGCAAGCTACCTGCTTTCTCTTTGCGCTTGCGTTTTTC
3781 -----+-----+-----+-----+-----+-----+-----+ 3840
      ATCGATAGCGGTACATTCCGGGTGACGTTTCGATGGACGAAAGAGAAACGCGAACGCAAAAG
      CCTTGTCAGATAGCCCAGTAGCTGACATTCATCCGGGGTCAGCACCGTTTCTGCGGACT
3841 -----+-----+-----+-----+-----+-----+-----+ 3900
      GGAACAGGTCATCGGGTCATCGACTGTAAGTAGGCCCCAGTCGTGGCAAAGACGCCTGA
      GGCTTTCTACGTGTTCCGCTTCCTTTAGCAGCCCTTGCGCCCTGAGTGCTTGCGGCAGCG
3901 -----+-----+-----+-----+-----+-----+-----+ 3960
      CCGAAAGATGCACAAGGCGAAGGAAATCGTCGGGAACGCGGGACTCACGAACGCCGTCGC

      |----- par locus -----
3961 TGAAGCTACATATATGTGATCCGGGCAAATCGCTGAATATTCCTTTTGTCTCCGACCATC 4020
      ACTTCGATGTATATACTAGGCCCCGTTTAGCGACTTATAAGGAAAACAGAGGCTGGTAG

      B
      c
      g
      I
      ----- par locus -----
4021 AGGCACCTGAGTCGCTGTCTTTTTCGTGACATTTCAGTTCGCTGCGCTCACGGCTCTGGCA 4080
      TCCGTGGACTCAGCGACAGAAAAAGCACTGTAAGTCAAGCGACGCGAGTGCCGAGACCGT
      ----- par locus -----

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FIG. 5H

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GTGAATGGGGGTAAATGGCACTACAGGCGCCTTTTATGGATTCATGCAAGGAACTACCC
4081 -----+-----+-----+-----+-----+-----+ 4140
CACTTACCCCCATTACCGTGATGTCCGCGGAAATACCTAAGTACGTTCTTTGATGGG

----- par locus -----
ATAATACAAGAAAAGCCCGTCACGGGCTTCTCAGGGCGTTTATGGCGGGTCTGCTATGT
4141 -----+-----+-----+-----+-----+-----+ 4200
TATTATGTTCTTTTCGGGCAGTGCCCGAAGAGTCCCGCAAAATACCGCCCAGACGATACA

----- par locus -----
GGTGCTATCTGACTTTTTGCTGTTTCAGCAGTTCCTGCCCTCTGATTTTCCAGTCTGACCA
4201 -----+-----+-----+-----+-----+-----+ 4260
CCACGATAGACTGAAAAACGACAAGTCGTCAAGGACGGGAGACTAAAAGGTCAGACTGGT

----- par locus -----
CTTCGGATTATCCCGTGACAGGTCATTTCAGACTGGCTAATGCACCCAGTAAGGCAGCGGT
4261 -----+-----+-----+-----+-----+-----+ 4320
GAAGCCTAATAGGGCACTGTCCAGTAAGTCTGACCGATTACGTGGGTCATTCCGTCGCCA

                                     N      B
                                     s      s
                                     i      a
                                     I      I
-----+-----+-----+-----+-----+-----+ 4380
4321 ATCATCAACAGGCTTACCCGTCTTACTGTCTGAAGACGTGCGTAACGTATGCATGGTCTCC
TAGTAGTTGTCCGAATGGGCAGAATGACAGCTTCTGCACGCATTGCATACGTACCAGAGG

                                     T1 hairpin
                                     ----->-----<-----
4381 CCATGCGAGAGTAGGGAAGTCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT
-----+-----+-----+-----+-----+-----+ 4440
GGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAGCTTTCTGA

-----+-----+-----+-----+-----+-----+
4441 GGGCCTTTCGTTTTATCTGTTGTTTGTCTGGTGAACGCTCTCCTGAGTAGGACAAATCCGC
-----+-----+-----+-----+-----+-----+ 4500
CCCGGAAAGCAAAATAGACAACAAACAGCCACTTGCGAGAGGACTCATCTGTTTAGGCG
-- T1 stop -->|

P
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4501 CGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCCGGAGGGTGGCGGGCAGGACGCCCGC
-----+-----+-----+-----+-----+-----+ 4560
GCCCTCGCCTAAACTTGCAACGCTTCGTTGCCGGGCTCCACCGCCCGTCCTGCGGGCG

                                     T2 hairpin
                                     ----->-----<-----
4561 CATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTCGCT
-----+-----+-----+-----+-----+-----+ 4620
GTATTTGACGGTCCGTAGTTTAAATTCGTCTTCCGGTAGGACTGCCTACCGGAAAAACGCA
----- T2 stop ---->|

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FIG. 5I

A
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4621 TTCTACAAACTCTTTTGTATTATTTTCTAAATACATTCAAATATGGACGTCGTACTTAACT
-----+-----+-----+-----+-----+-----+-----+ 4680
AAGATGTTTGTAGAAAACAAATAAAAAGATTTATGTAAGTTTATACCTGCAGCATGAATTG
* -

4681 TTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAATTCCTTTAGAAATACTTTGGCAGC
-----+-----+-----+-----+-----+-----+-----+ 4740
AAAATTTTCATACCCGTTAGTTAACGAGGACAATTTTAACGAAATCTTTATGAAACCGTCG
d * S K F Y P C D I A G T L I A K S I S Q C -
|<--- luxR protein ---

4741 GGTTCGTTGTATTGAGTTTCATTTGCGCATTGGTTAAATGGAAAGTGACCGTGGCCTTAC
-----+-----+-----+-----+-----+-----+-----+ 4800
CCAAACAACATAACTCAAAGTAAACGCGTAACCAATTTACCTTTCACTGGCAGCGGAATG
d R N T T N L K M Q A N T L H F T V T R K -

4801 TACAGCCTAATATTTTGAATATCCCAAGAGCTTTTTCCTTCGCATGCCACGCTAAAC
-----+-----+-----+-----+-----+-----+-----+ 4860
ATGTCGGATTATAAAACTTTATAGGGTTCTCGAAAAGGAAGCGTACGGGTGCGATTTG
d S C G L I K S I D W S S K G E C A W A L -

4861 ATTCCTTTTCTCTTTTGGTTAAATCGTTGTTTGATTTATTATTGCTATATTTATTTTC
-----+-----+-----+-----+-----+-----+-----+ 4920
TAAGAAAAGAGAAAACCAATTTAGCAACAACTAAATAATAAACGATATAAATAAAAAG
d C E K E R K T L D N N S K N N A I N I K -

4921 GATAATTATCAACTAGAGAAGGAACAATTAATGGTATGTTTCATACACGCATGTAAAAATA
-----+-----+-----+-----+-----+-----+-----+ 4980
CTATTAATAGTTGATCTCTTCCTTGTTAATTACCATAACAAGTATGTGCGTACATTTTAT
d R Y N D V L S P V I L P I N M C A H L F -

B
s
m
I

4981 AACTATCTATATAGTTGTCTTTCTCTGAATGTGCAAACTAAGCATTCGGAAGCCATTAT
-----+-----+-----+-----+-----+-----+-----+ 5040
TTGATAGATATATCAACAGAAAGAGACTTACACGTTTGTATTCGTAAGGCTTCGGTAATA
d L S D I Y N D K E S H A F S L M G F G N -

5041 TAGCAGTATGAATAGGGAACTAAACCCAGTGATAAGACCTGATGATTTGCTTCTTTAA
-----+-----+-----+-----+-----+-----+-----+ 5100
ATCGTCATACTTATCCCTTTGATTTGGGTCACTATTCTGGACTACTAAAGCGAAGAAATT
d N A T H I P F S F G T I L G S S K A E K -

5101 TTACATTTGGAGATTTTTTATTACAGCATTGTTTTCAAATATATTCCAATTAATCGGTG
-----+-----+-----+-----+-----+-----+-----+ 5160
AATGTAAACCTCTAAAAATAAATGTCGTAACAAAAGTTTATATAAGGTTAATTAGCCAC
d I V N P S K K N V A N N E F I N W N I P -

5161 AATGATTGGAGTTAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCAT
-----+-----+-----+-----+-----+-----+-----+ 5220
TTACTAACCTCAATCTTATTAGATGATATCCTAGTATAAAATAATTTAATCGCAGTAGTA
d S H N S N S Y D V I P D Y K I L N A D D -

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FIG. 5J

5221 AATATTGCCTCCATTTTTTAGGGTAATTATCCAGAATTGAAATATCAGATTTAACCATAG 5280
 -----+-----+-----+-----+-----+-----+-----+-----+
 TTATAACGGAGGTAAAAAATCCCATTAATAGGTCTTAACCTTTATAGTCTAAATTGGTATC
 d Y Y Q R W K K P Y N D L I S I D S K V M -

 N
 r
 u
 I
 5281 AATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTACCATTTTAGTCATATCAG 5340
 -----+-----+-----+-----+-----+-----+-----+
 TTACTCCTATTTACTAGCGCTCATTTATTATAAGTGTTACATGGTAAAATCAGTATAGTC
 S H P Y I I A L L Y Y E C H V M K T M D -

 5341 ATAAGCATTGATTAATATCATTATTGCTTCTACAGGCTTTAATTTTATTAATTATCTGT 5400
 -----+-----+-----+-----+-----+-----+-----+
 TATTCGTAACATAATTATAGTAATAACGAAGATGTCCGAAATTAAAATAATTAAAGACA
 S L C Q N I D N N S R C A K I K N I I R -

 5401 AAGTGTCGTCGGCATTATGTCTTTCATACCCATCTCTTTATCCTTACCTATTGTTTGTC 5460
 -----+-----+-----+-----+-----+-----+-----+
 TTCACAGCAGCCGTAAATACAGAAAGTATGGGTAGAGAAATAGGAATGGATAACAAACAG
 Y T D D A N I D K M
 <---- luxR protein ---|

 GCAAGTTTTCGCTGTTATATATCATTAAAACGGTAATAGATTGACATTTGATTCTAATAA
 5461 -----+-----+-----+-----+-----+-----+-----+ 5520
 CGTTCAAAACGCACAATATATAGTAATTTTGCCATTATCTAACTGTAAACTAAGATTATT
 <-----| <-----| <-----| <----- Promoter (luxPL) -----

 luxR mRNA start sites

 CRP Binding Site

 ATTGGATTTTGTACACTATTATATCGCTTGAAATACAATTGTTTAAACATAAGTACCTG
 5521 -----+-----+-----+-----+-----+-----+-----+ 5580
 TAACCTAAAAACAGTGTGATAATATAGCGAACTTTATGTTAACAAATTGTATTTCATGGAC

 ----- Promoter (luxPR) -----> C B
 lux operator site -35 -10 l b
 -----+-----+-----+-----+-----+-----+-----+ a a
 TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTATAGTCGATTAATCGATTGATT I I
 5581 -----+-----+-----+-----+-----+-----+-----+ 5640
 ATCCTAGCATGTCCAAATGCGTTCCTTTACCAACAATATCAGCTAATTAGCTAAACTAA
 |---- 1209-85 -----> |-- mRNA start -->

 NdeI
 |
 5641 CTAGATTTGTTTAACTAATTAAAGGAGGAATAACATATGATCGCTCCACCATGCACCAG 5700
 -----+-----+-----+-----+-----+-----+-----+
 GATCTAAACAAAATTGATTAATTTCTCCTTATTGTATACTAGCGAGGTGGTACGTGGTC

 b M I A P P C T S -
 |-- RANK -->

 5701 TGAGAAGCATTATGAGCATCTGGGACGGTGCTGTAACAAATGTGAACCAGGAAAGTACAT 5760
 -----+-----+-----+-----+-----+-----+-----+
 ACTCTTCGTAATACTCGTAGACCCTGCCACGACATTGTTTACACTTGGTCCTTTTCATGTA

 b E K H Y E H L G R C C N K C E P G K Y M -

FIG. 5K

5761 GTCTTCTAAATGCACTACTACCTCTGACAGTGTATGTCTGCCCTGTGGCCCGGATGAATA 5820
 CAGAAGATTTACGTGATGATGGAGACTGTACATACAGACGGGACACCGGGCCTACTTAT
 b S S K C T T T S D S V C L P C G P D E Y -
 CTTGGATAGCTGGAATGAAGAAGATAAATGCTTGCTGCATAAAGTTTGTGATACAGGCAA
 5821 5880
 GAACCTATCGACCTTACTTCTTCTATTACGAACGACGTATTTCAAACACTATGTCCGTT
 b L D S W N E E D K C L L H K V C D T G K -
 ApaLI
 5881 GGCCTGGTGGCCGTGGTCGCCGCAACAGTACGACCCCCCGGCGCTGCGCGTGCACGGC 5940
 CCGGGACCACCGGCACCAGCGGCCGTTGTCATGCTGGGGGGCCGCGACGCGCACGTGCCG
 b A L V A V V A G N S T T P R R C A C T A -
 KpnI
 Acc65I
 5941 TGGGTACCACTGGAGCCAGGACTGCGAGTGCTGCCGCCGCAACACCGAGTGC GCGCCGGG 6000
 ACCCATGGTGACCTCGGTCTCTGACGCTCACGACGGCGGCGTTGTGGCTACGCGCGGCCC
 b G Y H W S Q D C E C C R R N T E C A P G -
 6001 CCTGGGCGCCCAGCACCCGTTGCAGCTCAACAAGGACACAGTGTGCAAACCTTGCCTTGC 6060
 GGACCCGCGGGTCTGTTGGGCAACGTCGAGTTGTTCTGTGTACACGTTTGAACGGAACG
 b L G A Q H P L Q L N K D T V C K P C L A -
 6061 AGGCTACTTCTCTGATGCCCTTTTCTCCACGGACAAATGCAGACCCTGGACCAACTGTAC 6120
 TCCGATGAAGAGACTACGGAAAAGGAGGTGCCTGTTTACGTCTGGGACCTGGTTGACATG
 b G Y F S D A F S S T D K C R P W T N C T -
 6121 CTTCTTGGAAGAGAGTAGAACATCATGGGACAGAGAAATCCGATGTGGTTTGCAGTTC 6180
 GAAGGAACCTTTCTCTCATCTTGTAGTACCCTGTCTCTTTAGGCTACACCAAACGTCAAG
 b F L G K R V E H H G T E K S D V V C S S -
 AccI
 SalI
 6181 TTCTCTGCCAGCTAGAAAAACCAACCAATGAACCCCATGTTTACGTCGACAAAACCTCACAC 6240
 AAGAGACGGTGCATCTTTTGGTGGTTTACTTGGGGTACAAATGCAGCTGTTTGTAGTGTG
 b S L P A R K P P N E P H V Y V D K T H T -
 <-- end RANK --||--start Fc-->

BspEI | AhdI |
 6241 ATGTCCACCTTGTCCAGCTCCGGAACCTCTGGGGGACCGTCAGTCTTCCTCTTCCCCC 6300
 -----+-----+-----+-----+-----+
 TACAGGTGGAACAGGTCGAGGCCTTGAGGACCCCCCTGGCAGTCAGAAGGAGAAGGGGG
 b C P P C P A P E L L G G P S V F L F P P -
 BspHI |
 6301 AAAACCCAAGGACACCCATCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGA 6360
 -----+-----+-----+-----+-----+
 TTTTGGGTTCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCACCACCT
 b K P K D T L M I S R T P E V T C V V V D -
 CGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
 6361 -----+-----+-----+-----+-----+ 6420
 GCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGT
 b V S H E D P E V K F N W Y V D G V E V H -
 TAATGCCAAGACAAAGCCGCGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGT
 6421 -----+-----+-----+-----+-----+ 6480
 ATTACGGTTCCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCACACCAGTCGCA
 b N A K T K P R E E Q Y N S T Y R V V S V -
 EcoNI |
 6481 CCTCACCGTCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA 6540
 -----+-----+-----+-----+-----+
 GGAGTGGCAGGACGTGGTCTTGACCGACTTACCGTTCTCTCATGTTACAGTTCCAGAGGTT
 b L T V L H Q D W L N G K E Y K C K V S N -
 CAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGA
 6541 -----+-----+-----+-----+-----+ 6600
 GTTTCGGGAGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCCGTCGGGGCTCT
 b K A L P A P I E K T I S K A K G Q P R E -
 BsrGI | SmaI | SexAI |
 6601 ACCACAGGTGTACACCCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCT 6660
 -----+-----+-----+-----+-----+
 TGGTGTCCACATGTGGGACGGGGTAGGGCCCTACTCGACTGGTTCCTGGTCCAGTCGGA
 b P Q V Y T L P P S R D E L T K N Q V S L -
 GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGG
 6661 -----+-----+-----+-----+-----+ 6720
 CTGGACGGACCAGTTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCTCTCGTTACC
 b T C L V K G F Y P S D I A V E W E S N G -
 GCAGCCGGAACAACACTACAAGACCACGCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
 6721 -----+-----+-----+-----+-----+ 6780
 CGTCGGCCCTCTTGTGATGTTCTGGTGGCGAGGGCACGACCTGAGGCTGCCGAGGAAGAA

b

Q P E N N Y K T T P P V L D S D G S F F -

6781 CCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATG
-----+-----+-----+-----+-----+ 6840
GGAGATGTCGTTCCGAGTGGCACCTGTCTCGTCCACCGTCGTCCCCTTGCAGAAGAGTAC

b

L Y S K L T V D K S R W Q Q G N V F S C -

6841 CTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCC
-----+-----+-----+-----+-----+ 6900
GAGGCACTACGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGGAGAGGGACAGAGG

b

S V M H E A L H N H Y T Q K S L S L S P -

BamHI
|

6901 GGGTAAATAATGGATCCGCGGAAGAAGAAGAAGAAGAAGCCCGAAAGGAAGCTGA
-----+-----+-----+-----+-----+ 6960
CCCATTATTATTACCTAGGCGCCTTCTTCTTCTTCTTCTTCTTTCGGGCTTTCCTTCGACT

b

G K *

BlpI
|

T7 hairpin
-----> <---

6961 GTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGT
-----+-----+-----+-----+-----+ 7020
CAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGGGGAACCCGGAGATTGCCCCA
----->

<-----
7021 CTTGAGGGGTTTTTGTCTGAAAGGAGGAACCGCTCTTCACGCTCTTCACGCGGATAAATA
-----+-----+-----+-----+-----+ 7080
GAACTCCCCAAAAACGACTTTCCTCCTTGGCGAGAAGTGCAGAGAAGTGCGCCTATTAT
-T7 stop ---->|

toop hairpin
----->

7081 AGTAACGATCCGGTCCAGTAATGACCTCAGAACTCCATCTGGATTGTTCAGAACGCTCG
-----+-----+-----+-----+-----+ 7140
TCATTGCTAGGCCAGGTCATTACTGGAGTCTTGAGGTAGACCTAAACAAGTCTTGCGAGC

toop hairpin
<-----

7141 GTTGCCGCGCGGGCGTTTTTTATTGGTGAGAATCGCAGCAACTTGTCGCGCCAATCGAGCC
-----+-----+-----+-----+-----+ 7200
CAACGGCGGCGCGCAAAAAATAACCACTCTTAGCGTCGTTGAACAGCGCGGTTAGCTCGG
-- toop stop -->|

7201 ATGTCGTCGTCAACGACCCCCCATTCAGAACAGCAAGCAGCATTGAGAACTTTGGAATC
-----+-----+-----+-----+-----+ 7260
TACAGCAGCAGTTGCTGGGGGTAAGTTCTTGTCGTTTCGTCTGTAACCTCTTGAAACCTTAG

7261 CAGTCCCTCTTCCACCTGCTGACCG
-----+-----+-----+ 7285
GTCAGGGGAGAAGGTGGACGACTGGC

FIG. 6A

[AatII sticky end] 5' GCGTAACGTATGCATGGTCTCC-
(position #4358 in pAMG21) 3' TGCACGCATTGCATACGTACCAGAGG-

-CCATGCGAGAGTAGGGAAC TGCCAGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACT-
-GGTACGCTCTCATCCCTTGACGGTCCGTAGTTTATTTTGCTTTCCGAGTCAGCTTTCTGA-

-GGGCCTTTCGTTTTATCTGTTGTTTGTCTGGTGAACGCTCTCCTGAGTAGGACAAATCCGC-
-CCCGGAAAGCAAAATAGACAACAAACAGCCACTTGCAGAGGACTCATCCTGTTTAGGCG-

-CGGGAGCGGATTTGAACGTTGCGAAGCAACGGCCCGAGGGTGGCGGGCAGGACGCCCCG-
-GCCCTCGCCTAAACTTGCAACGCTTCGTTGCCGGGCTCCACCGCCCGTCTGCGGGCG-

-CATAAACTGCCAGGCATCAAATTAAGCAGAAGGCCATCCTGACGGATGGCCTTTTTCGCT-
-GTATTTGACGGTCCGTAGTTTAATTCGTCTCCGGTAGGACTGCCTACCGGAAAAACGCA-

AatII

-TTCTACAAACTCTTTTGTTTATTTTCTAAATACATTCAAATATGGACGTCGTACTTAAC-
-AAGATGTTTGAGAAAACAAATAAAAAGATTATGTAAGTTTATACCTGCAGCATGAATTG-

-TTTTAAAGTATGGGCAATCAATTGCTCCTGTTAAATATGCTTTAGAAATACTTTGGCAGC-
-AAAATTCATACCCGTTAGTTAACGAGGACAATTTAACGAAATCTTTATGAAACCGTCG-

-GGTTTGTTGTTATGAGTTTCATTTGCGCATTTGGTTAAATGGAAAGTGACCGTGCGCTTAC-
-CCAAACAACATAACTCAAAGTAAACGCGTAACCAATTTACCTTTCAGTGGCAGCGAATG-

-TACAGCCTAATATTTTGAATATCCCAAGAGCTTTTTCCTTCGCATGCCCACGCTAAAC-
-ATGTCGGATTATAAAAACCTTTATAGGGTTCTCGAAAAAGGAAGCGTACGGGTGCGATTG-

-ATTCTTTTCTCTTTTGGTTAAATCGTTGTTGATTTATTATTTGCTATATTTATTTTTC-
-TAAGAAAAAGAGAAAACCAATTTAGCAACAACTAAATAATAACGATATAAATAAAAAG-

-GATAATTATCAACTAGAGAAGGAACAATTAATGGTATGTTTCATACACGCATGTAAAAATA-
-CTATTAATAGTTGATCTCTTCCTTGTTAATTACCATACAAGTATGTGCGTACATTTTAT-

-AATATCTATATAGTTGCTTTCTCTGAATGTGCAAACTAAGCATTCCGAAGCCATTAT-
-TTGATAGATATATCAACAGAAAGAGACTTACACGTTTGTATTCGTAAGGCTTCGGTAATA-

-TAGCAGTATGAATAGGGAACTAAACCCAGTGATAAGACCTGATGATTTTCGCTTCTTTAA-
-ATCGTCATACCTATCCCTTTGATTTGGGTCACTATCTTGACTACTAAAGCGAAGAAATT-

-TTACATTTGGAGATTTTTTATTTACAGCATTGTTTTCAAATATATTCCAATTAATCGGTG-
-AATGTAAACCTCTAAAAAATAAATGTCGTAACAAAAGTTTATATAAGGTAAATTAGCCAC-

-AATGATTGGAGTTAGAATAATCTACTATAGGATCATATTTTATTAAATTAGCGTCATCAT-
-TTACTAACCTCAATCTTATTAGATGATATCCTAGTATAAAATAATTTAATCGCAGTAGTA-

-AATATGCGCTCCATTTTTTAGGGTAATTATCCAGAATTGAAATATCAGATTTAACCATAG-
-TTATAACGGAGGTAAAAAATCCCATTAATAGGTCTTAACCTTATAGTCTAAATTGGTATC-

-AATGAGGATAAATGATCGCGAGTAAATAATATTCACAATGTACCATTTTAGTCATATCAG-
-TTACTCCTATTTACTAGCGCTCATTTATTATAAGTGTTACATGGTAAAAACAGTATAGTC-

-ATAAGCATTGATTAATATCATTTATTGCTTCTACAGGCTTTAATTTTATTAATTATCTGT-
-TATTCGTAACCTAATTATAGTAATAACGAAGATGTCGAAATTAATAATTAATAAGACA-

-AAGTGTCGTCGGCATTTATGCTTTTCATACCCATCTCTTTATCCTTACCTATTGTTTGTC-
-TTCACAGCAGCCGTAAATACAGAAAGTATGGGTAGAGAAATAGGAATGGATAACAAACAG-

-GCAAGTTTTGCGTGTATATATCATTTAAACGGTAATAGATTGACATTTGATTCTAATAA-
-CGTTCAAAACGCACAATATATAGTAATTTGCCATTATCTAACTGTAAACTAAGATTATT-

FIG. 6B

-ATTGGATTTTGTGACACTATTATATCGCTTGAAATACAATTGTTTAACATAAGTACCTG-
-TAACCTAAAAACAGTGTGATAATATAGCGAACTTTATGTTAACAAATTGTATTCATGGAC-
-TAGGATCGTACAGGTTTACGCAAGAAAATGGTTTGTATAGTCGATTAATCGATTTGATT-
-ATCCTAGCATGTCCAAATGCGTTCCTTTTACCAAACAATATCAGCTAATTAGCTAAACTAA-
-CTAGATTTGTTTTAACTAATTAAAGGAGGAATAACATATGGTTAACGCGTTGGAATTCGA-
-GATCTAAACAAAATTGATTAATTTCCCTCCTTATTGTATACCAATTGCGCAACCTTAAGCT-
-GCTCACTAGTGTGCGACCTGCAGGGTACCATGGAAGCTTACTCGAGGATCCGCGGAAAGAA-
-CGAGTGATCACAGCTGGACGTCCCATGGTACCTTCGAATGAGCTCCTAGGCGCCTTTCTT-
-GAAGAAGAAGAAGAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATA-
-CTTCTTCTTCTTCTTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTAT-
-ACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTTGCTGAAAGGAGG-
-TGATCGTATTGGGGAACCCCGGAGATTTGCCCAGAACTCCCCAAAAACGACTTTCTCTCC-
-AACCGCTCTTCACGCTCTTCACGC 3' [SacII sticky end]
-TTGGCGAGAAGTGCGAGAAGTG 5' (position #5904 in pAMG21)

FIG. 7

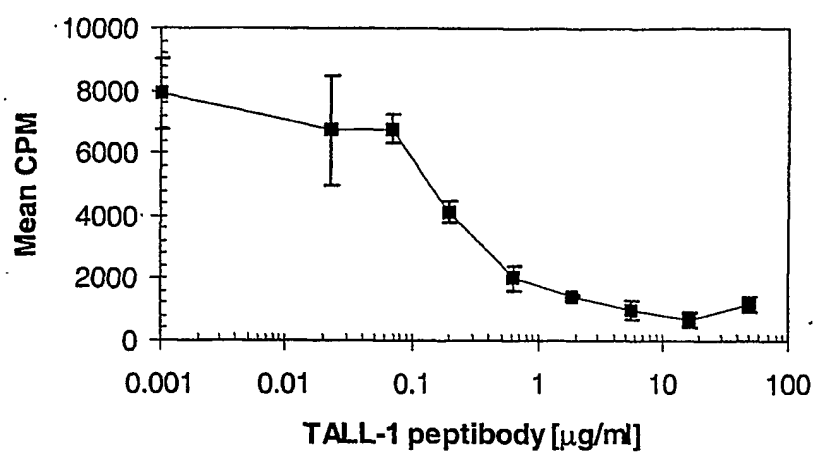


FIG. 8

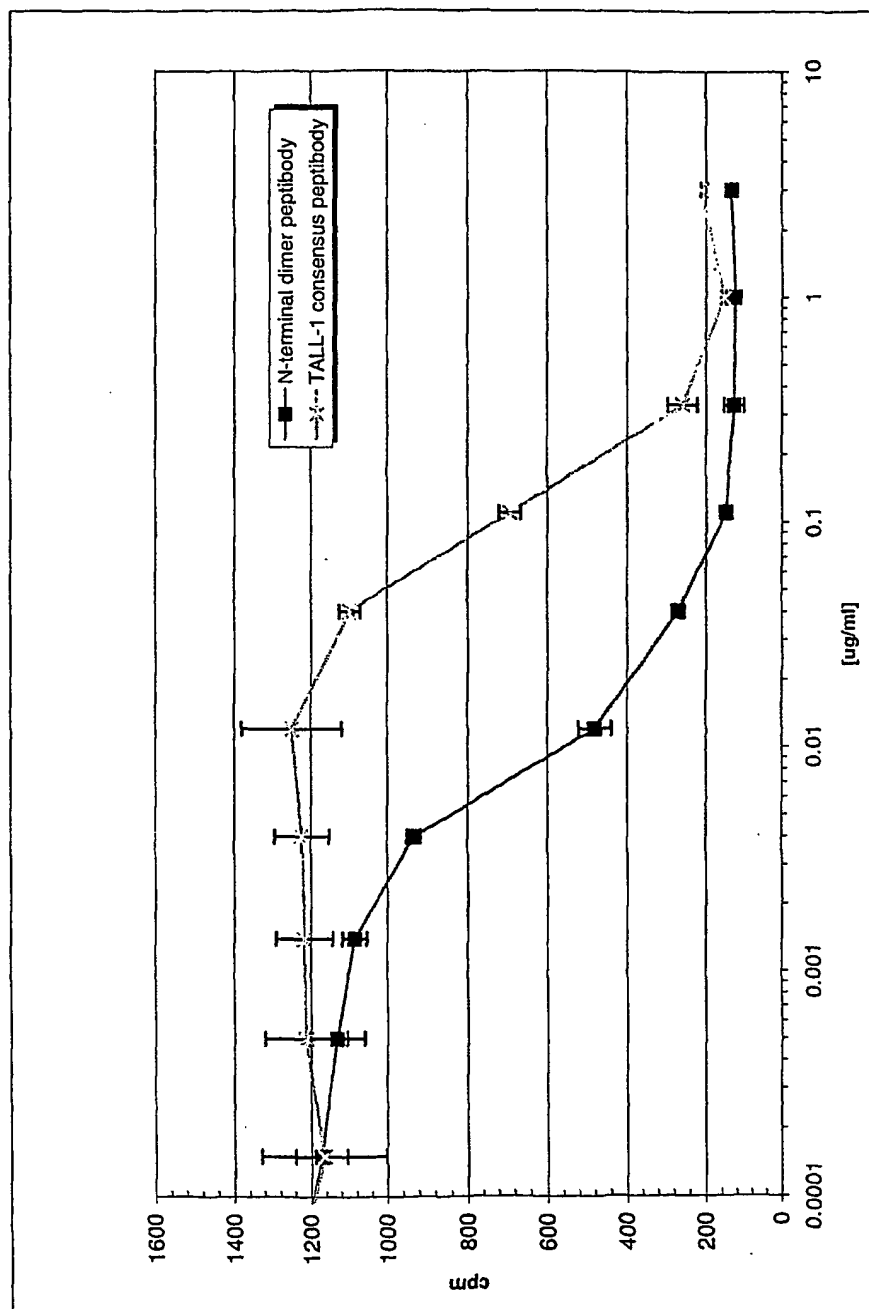


FIG. 9

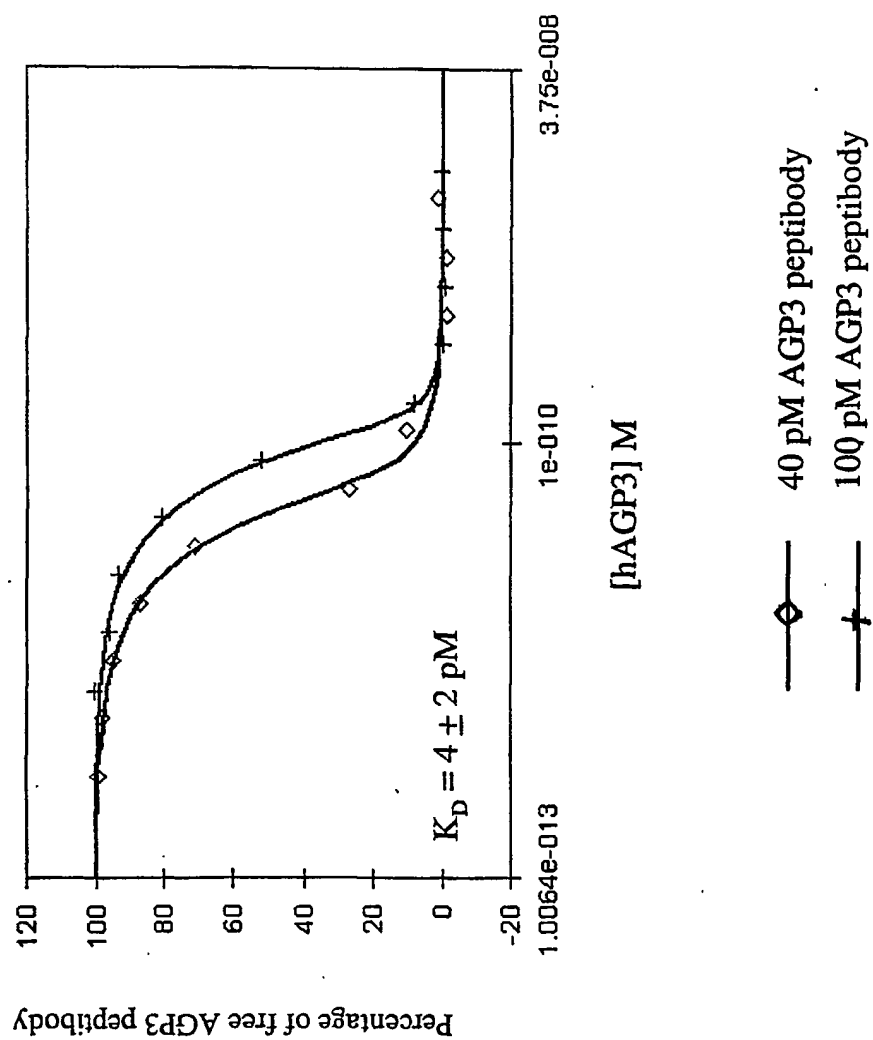


FIG. 10A

Against human AGP3

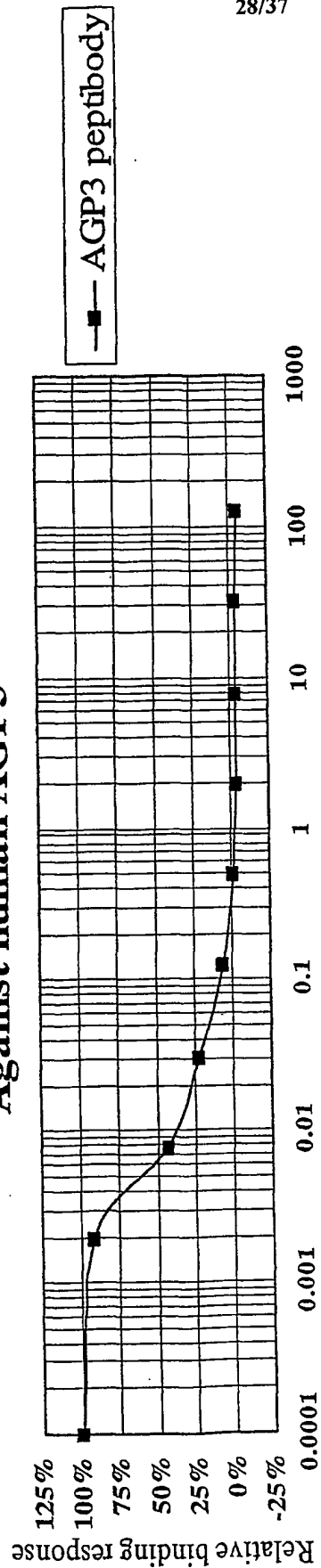
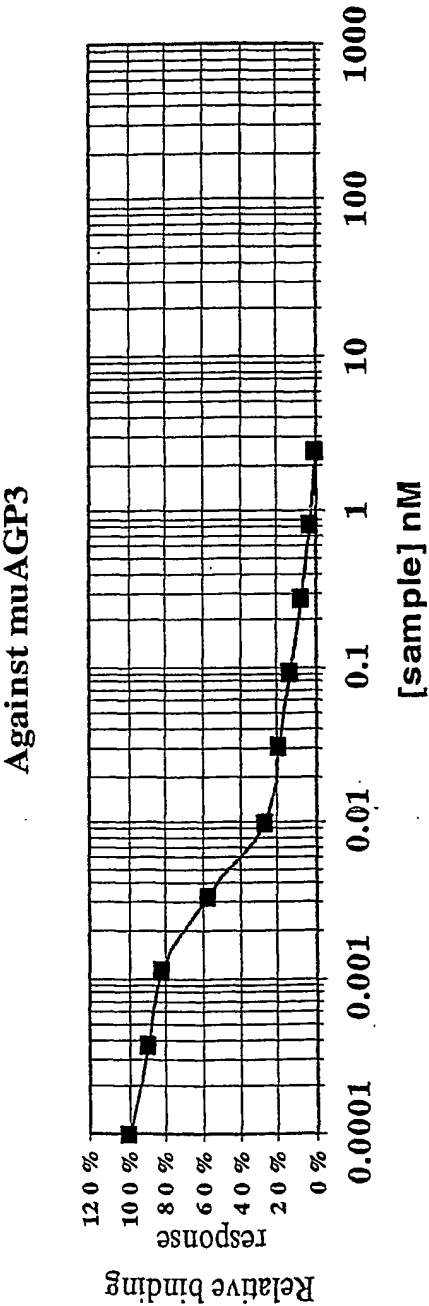


FIG. 10B



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FIG. 11A

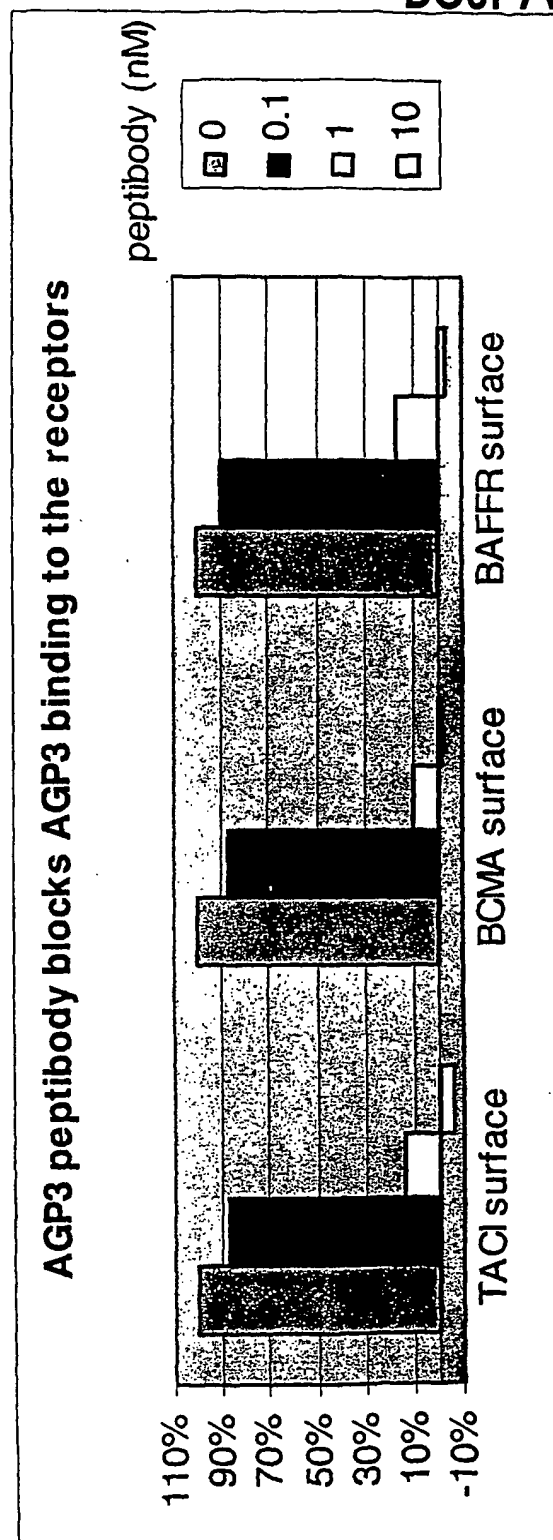
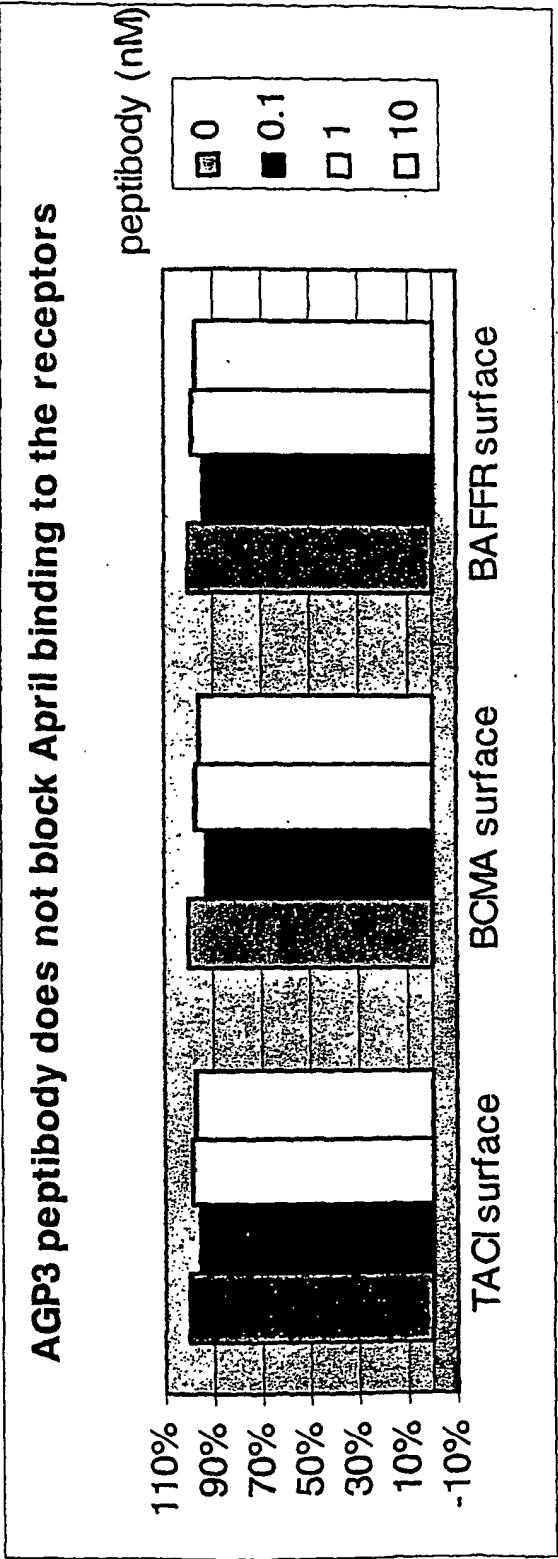


FIG. 11B



Best Available Copy

FIG. 12B

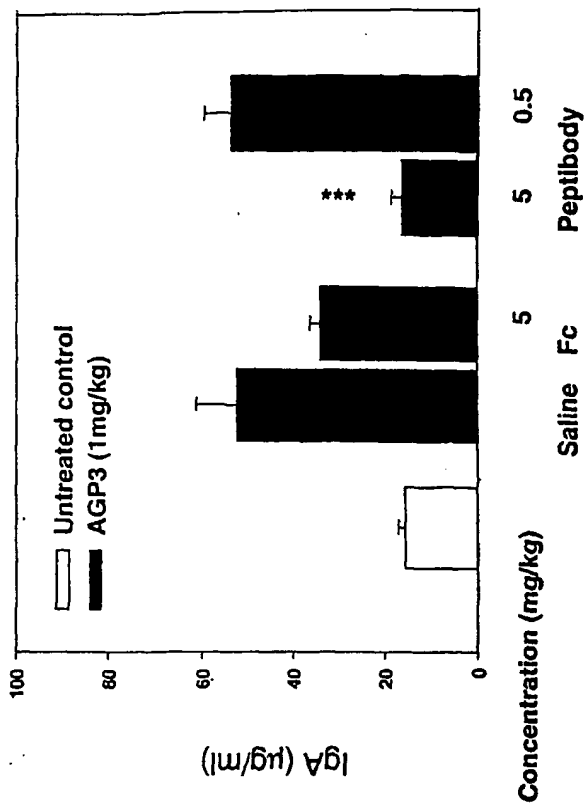


FIG. 12A

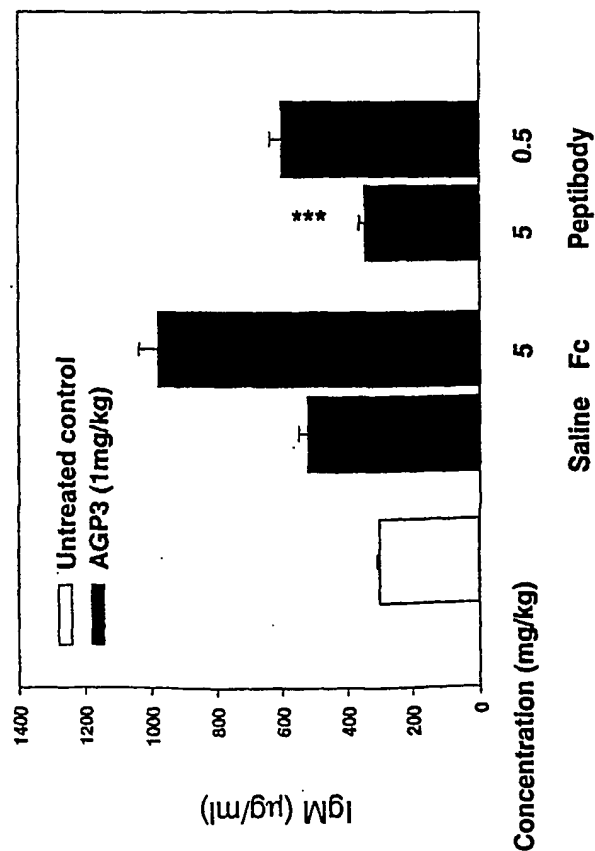
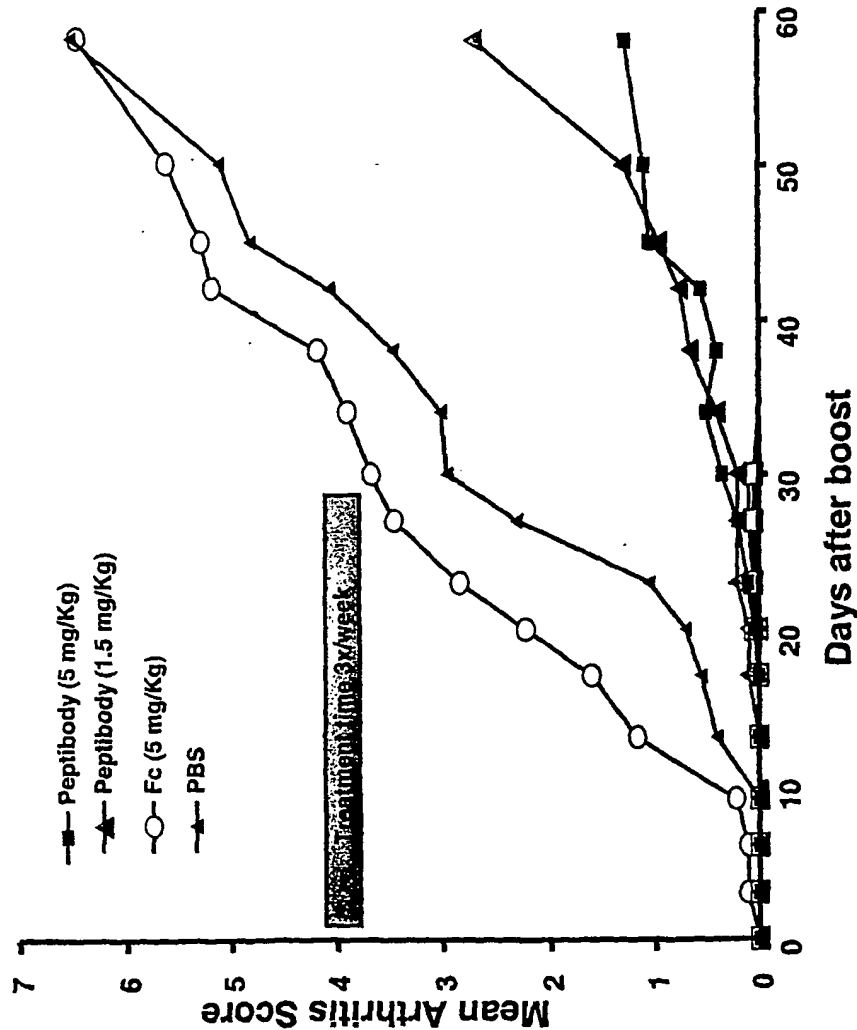


FIG. 13

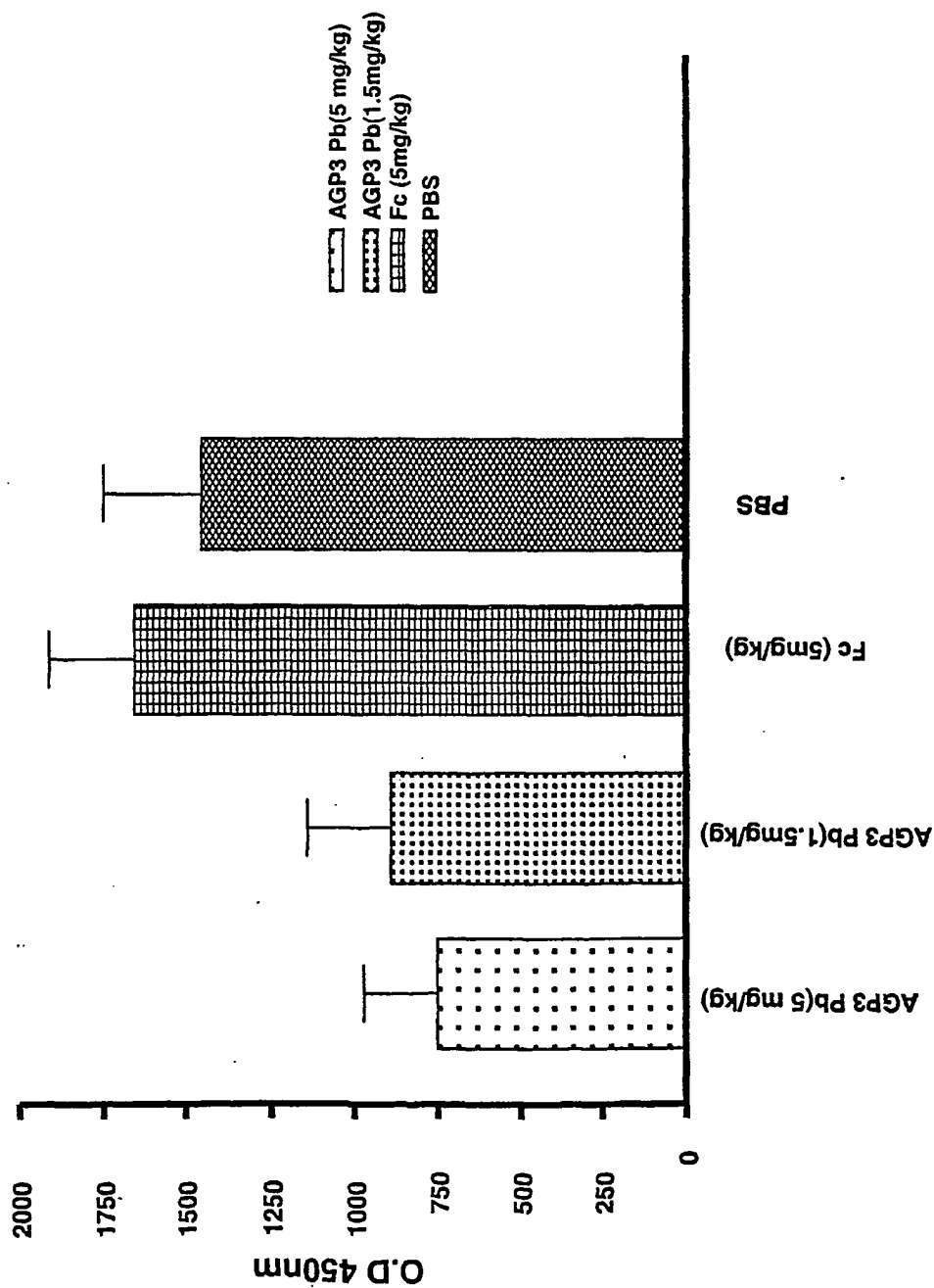


Time-to-Disease	Pb (5mg/Kg)	Pb (1.5mg/Kg)
P-value vs PBS	<0.0001	0.0001
P-value vs Fc	<0.0001	0.0004

Note: p-value based on log-rank test

FIG. 14

Reduced anti-collagen IgG2b upon
treatment with AGP3 peptibody

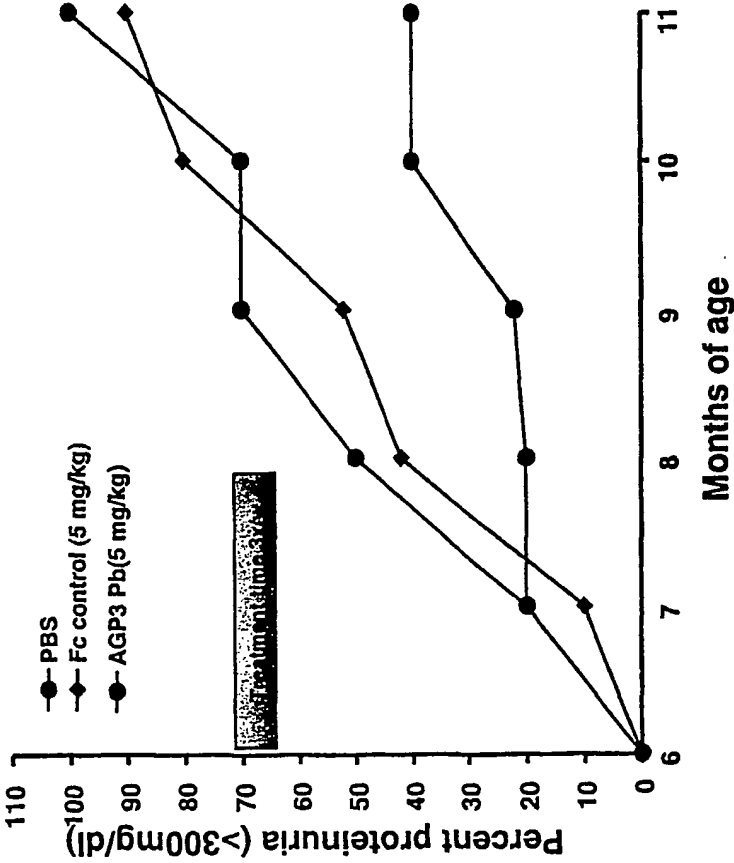


Serum samples were taken one week after final treatment of reagent (day 35).

The graph above is representative of the IgG1, IgG3, and IgG2a isotypes as well.

Fig. 15A

Delayed proteinuria with AGP3 protein blockers

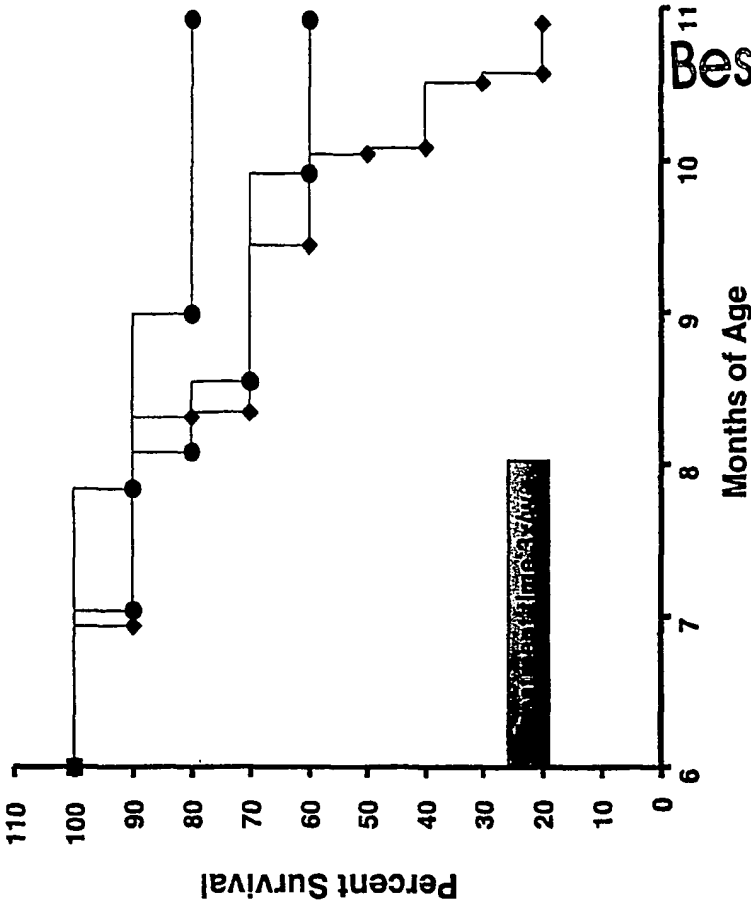


Proteinuria Incidence	Pb
p-value vs PBS	0.0108
P-vs Fc	0.0573

P-value based Fisher's Exact test

Fig. 15B

Prolonged survival with AGP3 blockers



Time-to-Death	Pb
p-value vs PBS	0.3685
p-value vs Fc	0.0159

P-value based log-rank test

Best Available Copy

FIG. 16A

BamHI
|

1 ATGCTTCCAGGCTGCAAGTGGGATCTTCTTATTAAGCAATGGGTATGCGATCCACTTGGA
-----+-----+-----+-----+-----+ 60
TACGAAGGTCCGACGTTACCCCTAGAAGAATAATTCGTTACCCATACGCTAGGTGAACCT

M L P G C K W D L L I K Q W V C D P L G -

61 TCCGGTTCTGCTACTGGTGGTTCCGGCTCCACCGCAAGCTCTGGTTCAGGCAGTGCGACT
-----+-----+-----+-----+-----+ 120
AGGCCAAGACGATGACCACCAAGGCCGAGGTGGCGTTCGAGACCAAGTCCGTCACGCTGA

S G S A T G G S G S T A S S G S G S A T -

NdeI
|

121 CATATGCTGCCGGGTGTAAATGGGACCTGCTGATCAAACAGTGGGTTTGTGACCCGCTG
-----+-----+-----+-----+-----+ 180
GTATACGACGGCCCAACATTTACCCTGGACGACTAGTTTGTACCCAAACACTGGGCGAC

H M L P G C K W D L L I K Q W V C D P L -

Sali
|

181 GGTGGAGGCGGTGGGTCGACAAACTCACACATGTCCACCTTGTCCAGCTCCGGAATC
-----+-----+-----+-----+-----+ 240
CCACCTCCGCCACCCAGCTGTTTTGAGTGTGTACAGGTGGAACAGGTCGAGGCCTTGAG

G G G G G V D K T H T C P P C P A P E L -

241 CTGGGGGACCGTCAGTCTTCCTCTTCCCCCAAACCAAGGACACCCTCATGATCTCC
-----+-----+-----+-----+-----+ 300
GACCCCCCTGGCAGTCAGAAGGAGAAGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGG

L G G P S V F L F P P K P K D T L M I S -

301 CGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAG
-----+-----+-----+-----+-----+ 360
GCCTGGGGACTCCAGTGTACGCACCAACACCTGCACTCGGTGCTTCTGGGACTCCAGTTC

R T P E V T C V V V D V S H E D P E V K -

361 TTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAG
-----+-----+-----+-----+-----+ 420
AAGTTGACCATGCACCTGCCGCACCTCCACGTATTACGGTTCGTTCGGCGCCCTCCTC

F N W Y V D G V E V H N A K T K P R E E -

421 CAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTG
-----+-----+-----+-----+-----+ 480
GTCATGTTGTCGTGCATGGCACACCAGTCGCAGGAGTGGCAGGACGTGGTCTTGACCGAC

Q Y N S T Y R V V S V L T V L H Q D W L -

FIG. 16B

AATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAA
481 -----+-----+-----+-----+-----+-----+ 540
TTACCGTTCCCTCATGTTACGTTCCAGAGGTTGTTTCGGGAGGGTCGGGGGTAGCTCTTT
N G K E Y K C K V S N K A L P A P I E K -
ACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCC
541 -----+-----+-----+-----+-----+ 600
TGGTAGAGGTTTCGGTTTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGGTAGG
T I S K A K G Q P R E P Q V Y T L P P S -
CGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC
601 -----+-----+-----+-----+-----+ 660
GCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGG
R D E L T K N Q V S L T C L V K G F Y P -
AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACAACACTACAAGACCACG
661 -----+-----+-----+-----+-----+ 720
TCGCTGTAGCGGCACCTCACCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGC
S D I A V E W E S N G Q P E N N Y K T T -
CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAG
721 -----+-----+-----+-----+-----+ 780
GGAGGGCACGACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTGAGTGGCACCTGTTT
P P V L D S D G S F F L Y S K L T V D K -
AGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAAC
781 -----+-----+-----+-----+-----+ 840
TCGTCCACCGTCGTCCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGAGACGTGTTG
S R W Q Q G N V F S C S V M H E A L H N -
CACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATAA
841 -----+-----+-----+-----+ 882
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ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc	96
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
20 25 30	

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Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
35 40 45	

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag	192
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	
50 55 60	

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg	240
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	
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Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn	
85 90 95	

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Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro	
100 105 110	

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag	384
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln	
115 120 125	

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc	432
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val	
130 135 140	

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Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val	

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ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc				576
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr				
	180	185	190	
gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg				624
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val				
	195	200	205	
atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg				672
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu				
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Ser Pro Gly Lys				
225				

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			20					25					30		
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
		35					40					45			
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
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Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
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Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
				85					90					95	
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro
			100					105					110		
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln
		115					120					125			
Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val
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Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
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A-743 PCT.ST25.txt

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atgtcgtcgt caacgacccc ccattcaaga acagcaagca gcattgagaa ctttggaatc 7260
cagtcctctt tccacctgct gaccg 7285

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<211> 7285

<212> PRT

<213> Artificial Sequence

<220>

<223> pAMG21-RANK-Fc vector

<220>

<221> misc_feature

<223> Xaa (Pos1,2,3,15,16,17) are each independently absent or amino acid residues;

Xaa (Pos5,6,7,9,13) are each independently amino acid residues.

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Cys Cys Thr Thr Cys Gly Cys Gly Thr Thr Gly Cys Thr Cys Ala Gly
 35 40 45

Thr Thr Gly Thr Cys Cys Ala Ala Cys Cys Cys Cys Gly Gly Ala Ala
 50 55 60

Ala Cys Gly Gly Gly Ala Ala Ala Ala Ala Gly Cys Ala Ala Gly Thr
 65 70 75 80

A-743 PCT.ST25.txt

Thr Thr Thr Cys Cys Cys Cys Gly Cys Thr Cys Cys Cys Gly Gly Cys
 85 90 95

Gly Thr Thr Thr Cys Ala Ala Thr Ala Ala Cys Thr Gly Ala Ala Ala
 100 105 110

Ala Cys Cys Ala Thr Ala Cys Thr Ala Thr Thr Thr Cys Ala Cys Ala
 115 120 125

Gly Thr Thr Thr Ala Ala Ala Thr Cys Ala Cys Ala Thr Thr Ala Ala
 130 135 140

Ala Cys Gly Ala Cys Ala Gly Thr Ala Ala Thr Cys Cys Cys Cys Gly
 145 150 155 160

Thr Thr Gly Ala Thr Thr Thr Gly Thr Gly Cys Gly Cys Cys Ala Ala
 165 170 175

Cys Ala Cys Ala Gly Ala Thr Cys Thr Thr Cys Gly Thr Cys Ala Cys
 180 185 190

Ala Ala Thr Thr Cys Thr Cys Ala Ala Gly Thr Cys Gly Cys Thr Gly
 195 200 205

Ala Thr Thr Thr Cys Ala Ala Ala Ala Ala Cys Thr Gly Thr Ala
 210 215 220

Gly Thr Ala Thr Cys Cys Thr Cys Thr Gly Cys Gly Ala Ala Ala Cys
 225 230 235 240

Gly Ala Thr Cys Cys Cys Thr Gly Thr Thr Thr Gly Ala Gly Thr Ala
 245 250 255

Thr Thr Gly Ala Gly Gly Ala Gly Gly Cys Gly Ala Gly Ala Thr Gly
 260 265 270

Thr Cys Gly Cys Ala Gly Ala Cys Ala Gly Ala Ala Ala Thr Gly
 275 280 285

Cys Ala Gly Thr Gly Ala Cys Thr Thr Cys Cys Thr Cys Ala Thr Thr
 290 295 300

Gly Ala Gly Thr Cys Ala Ala Ala Ala Gly Cys Gly Gly Thr Thr Thr
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 325 330 335

Cys Thr Ala Thr Gly Ala Cys Thr Gly Ala Cys Thr Cys Thr Gly Ala
 340 345 350

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Gly Ala Ala Ala Cys Ala Ala Ala Thr Gly Gly Cys Cys Gly Thr Thr
 355 360 365

Gly Thr Thr Gly Cys Ala Ala Gly Ala Ala Ala Ala Cys Gly Thr Cys
 370 375 380

Thr Thr Ala Cys Ala Cys Ala Cys Ala Ala Ala Gly Ala Gly Ala Thr
 385 390 395 400

Ala Ala Ala Ala Gly Thr Thr Thr Thr Thr Gly Thr Cys Ala Ala Ala
 405 410 415

Ala Ala Thr Cys Cys Thr Cys Thr Gly Ala Ala Gly Gly Ala Thr Cys
 420 425 430

Thr Cys Ala Thr Gly Gly Thr Thr Gly Ala Gly Thr Ala Cys Thr Gly
 435 440 445

Cys Gly Ala Gly Ala Gly Ala Gly Ala Gly Gly Gly Gly Ala Thr Ala
 450 455 460

Ala Cys Ala Cys Ala Gly Gly Cys Thr Cys Ala Gly Thr Thr Cys Gly
 465 470 475 480

Thr Thr Gly Ala Gly Ala Ala Ala Ala Thr Cys Ala Thr Cys Ala Ala
 485 490 495

Ala Gly Ala Thr Gly Ala Ala Cys Thr Gly Cys Ala Ala Ala Gly Ala
 500 505 510

Cys Thr Gly Gly Ala Thr Ala Thr Ala Cys Thr Ala Ala Ala Gly Thr
 515 520 525

Ala Ala Ala Gly Ala Cys Thr Thr Thr Ala Cys Thr Thr Thr Gly Thr
 530 535 540

Gly Gly Cys Gly Thr Ala Gly Cys Ala Thr Gly Cys Thr Ala Gly Ala
 545 550 555 560

Thr Thr Ala Cys Thr Gly Ala Thr Cys Gly Thr Thr Thr Ala Ala Gly
 565 570 575

Gly Ala Ala Thr Thr Thr Thr Gly Thr Gly Gly Cys Thr Gly Gly Cys
 580 585 590

Cys Ala Cys Gly Cys Cys Gly Thr Ala Ala Gly Gly Thr Gly Gly Cys
 595 600 605

Ala Ala Gly Gly Ala Ala Cys Thr Gly Gly Thr Thr Cys Thr Gly Ala
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A-743 PCT.ST25.txt

Thr Gly Thr Gly Gly Ala Thr Thr Thr Ala Cys Ala Gly Gly Ala Gly
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Cys Cys Ala Gly Ala Ala Ala Ala Gly Cys Ala Ala Ala Ala Ala Cys
645 650 655

Cys Cys Cys Gly Ala Thr Ala Ala Thr Cys Thr Thr Cys Thr Thr Cys
660 665 670

Ala Ala Cys Thr Thr Thr Thr Gly Cys Gly Ala Gly Thr Ala Cys Gly
675 680 685

Ala Ala Ala Ala Gly Ala Thr Thr Ala Cys Cys Gly Gly Gly Gly Cys
690 695 700

Cys Cys Ala Cys Thr Thr Ala Ala Ala Cys Cys Gly Thr Ala Thr Ala
705 710 715 720

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725 730 735

Thr Gly Cys Gly Gly Gly Gly Ala Gly Thr Ala Thr Ala Gly Thr Thr
740 745 750

Ala Thr Ala Thr Gly Cys Cys Cys Gly Gly Ala Ala Ala Ala Gly Thr
755 760 765

Thr Cys Ala Ala Gly Ala Cys Thr Thr Cys Thr Thr Thr Cys Thr Gly
770 775 780

Thr Gly Cys Thr Cys Gly Cys Thr Cys Cys Thr Thr Cys Thr Gly Cys
785 790 795 800

Gly Cys Ala Thr Thr Gly Thr Ala Ala Gly Thr Gly Cys Ala Gly Gly
805 810 815

Ala Thr Gly Gly Thr Gly Thr Gly Ala Cys Thr Gly Ala Thr Cys Thr
820 825 830

Thr Cys Ala Cys Cys Ala Ala Ala Cys Gly Thr Ala Thr Thr Ala Cys
835 840 845

Cys Gly Cys Cys Ala Gly Gly Thr Ala Ala Ala Gly Ala Ala Cys Cys
850 855 860

Cys Gly Ala Ala Thr Cys Cys Gly Gly Thr Gly Thr Thr Thr Ala Cys
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885 890 895

A-743 PCT.ST25.txt

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 915 920 925

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 930 935 940

Ala Cys Thr Thr Cys Cys Cys Gly Thr Thr Thr Thr Gly Ala Thr Thr
 945 950 955 960

Thr Cys Gly Cys Cys Ala Thr Thr Cys Ala Thr Gly Thr Gly Gly Cys
 965 970 975

Gly Cys Ala Cys Gly Cys Cys Cys Gly Thr Thr Cys Gly Cys Gly Thr
 980 985 990

Gly Ala Thr Cys Thr Gly Cys Gly Thr Cys Gly Cys Cys Gly Thr Ala
 995 1000 1005

Thr Gly Cys Cys Ala Cys Cys Ala Gly Thr Gly Cys Thr Gly Cys
 1010 1015 1020

Gly Thr Cys Gly Thr Cys Gly Gly Gly Cys Thr Ala Thr Thr Gly
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 1040 1045 1050

Gly Gly Cys Thr Gly Thr Gly Thr Thr Thr Cys Cys Ala Cys Thr
 1055 1060 1065

Ala Thr Gly Ala Cys Cys Cys Gly Cys Thr Gly Gly Cys Cys Ala
 1070 1075 1080

Ala Cys Cys Gly Cys Gly Thr Cys Cys Ala Gly Thr Gly Cys Thr
 1085 1090 1095

Cys Cys Ala Thr Cys Ala Cys Cys Ala Cys Gly Cys Thr Gly Gly
 1100 1105 1110

Cys Cys Ala Thr Thr Gly Ala Gly Thr Gly Cys Gly Gly Ala Cys
 1115 1120 1125

Thr Gly Gly Cys Gly Ala Cys Gly Gly Ala Gly Thr Cys Thr Gly
 1130 1135 1140

Cys Thr Gly Cys Cys Gly Gly Ala Ala Ala Ala Cys Thr Cys Thr
 1145 1150 1155

A-743 PCT.ST25.txt

Cys Cys Ala Thr Cys Ala Cys Cys Cys Gly Thr Gly Cys Cys Ala
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 1175 1180 1185
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 1190 1195 1200
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 1220 1225 1230
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 1235 1240 1245
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 1280 1285 1290
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 1295 1300 1305
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 1310 1315 1320
 Cys Gly Cys Gly Cys Cys Gly Cys Ala Gly Cys Cys Gly Thr Gly
 1325 1330 1335
 Thr Gly Gly Thr Ala Thr Gly Gly Gly Ala Ala Ala Ala Cys Ala
 1340 1345 1350
 Ala Ala Cys Ala Ala Cys Gly Cys Ala Ala Ala Ala Ala Gly Cys
 1355 1360 1365
 Ala Gly Gly Gly Cys Thr Gly Gly Ala Thr Ala Cys Cys Cys
 1370 1375 1380
 Thr Gly Gly Gly Cys Ala Thr Gly Gly Ala Thr Gly Ala Ala Cys
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A-743 PCT.ST25.txt

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 1415 1420 1425
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 1445 1450 1455
 Ala Gly Thr Cys Cys Cys Gly Thr Gly Gly Ala Ala Thr Ala Ala
 1460 1465 1470
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 1475 1480 1485
 Gly Thr Cys Gly Thr Gly Ala Thr Gly Cys Gly Gly Ala Cys Ala
 1490 1495 1500
 Gly Gly Gly Ala Ala Cys Gly Thr Cys Ala Gly Gly Ala Thr Ala
 1505 1510 1515
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 1520 1525 1530
 Ala Ala Cys Gly Gly Cys Ala Gly Cys Thr Gly Ala Cys Gly Cys
 1535 1540 1545
 Gly Cys Gly Ala Ala Ala Thr Cys Gly Cys Gly Gly Ala Ala Gly
 1550 1555 1560
 Gly Gly Cys Gly Cys Thr Thr Cys Ala Cys Thr Gly Cys Cys Ala
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 Ala Thr Cys Gly Thr Gly Ala Gly Gly Cys Gly Gly Thr Ala Ala
 1580 1585 1590
 Ala Ala Cys Gly Cys Gly Ala Ala Gly Thr Thr Gly Ala Gly Cys
 1595 1600 1605
 Gly Thr Cys Gly Thr Gly Thr Gly Ala Ala Gly Gly Ala Gly Cys
 1610 1615 1620
 Gly Cys Ala Thr Gly Ala Thr Thr Cys Thr Gly Thr Cys Ala Cys
 1625 1630 1635
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A-743 PCT.ST25.txt

Cys Thr Thr Cys Cys Cys Cys Cys Thr Gly Ala Ala Ala Gly Thr
 1670 1675 1680
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 1685 1690 1695
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 1700 1705 1710
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 1715 1720 1725
 Cys Thr Gly Ala Ala Gly Cys Cys Cys Gly Ala Cys Ala Gly Cys
 1730 1735 1740
 Gly Cys Ala Cys Ala Ala Ala Ala Ala Ala Thr Cys Ala Gly Cys
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 1760 1765 1770
 Cys Ala Ala Cys Cys Thr Cys Ala Thr Cys Ala Thr Cys Cys Ala
 1775 1780 1785
 Gly Cys Thr Thr Cys Thr Gly Gly Thr Gly Cys Ala Thr Cys Cys
 1790 1795 1800
 Gly Gly Cys Cys Cys Cys Cys Cys Cys Thr Gly Thr Thr Thr Thr
 1805 1810 1815
 Cys Gly Ala Thr Ala Cys Ala Ala Ala Ala Cys Ala Cys Gly Cys
 1820 1825 1830
 Cys Thr Cys Ala Cys Ala Gly Ala Cys Gly Gly Gly Gly Ala Ala
 1835 1840 1845
 Thr Thr Thr Thr Gly Cys Thr Thr Ala Thr Cys Cys Ala Cys Ala
 1850 1855 1860
 Thr Thr Ala Ala Ala Cys Thr Gly Cys Ala Ala Gly Gly Gly Ala
 1865 1870 1875
 Cys Thr Thr Cys Cys Cys Cys Ala Thr Ala Ala Gly Gly Thr Thr
 1880 1885 1890
 Ala Cys Ala Ala Cys Cys Gly Thr Thr Cys Ala Thr Gly Thr Cys
 1895 1900 1905
 Ala Thr Ala Ala Ala Gly Cys Gly Cys Cys Ala Thr Cys Cys Gly
 1910 1915 1920

A-743 PCT.ST25.txt

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 1940 1945 1950
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 1955 1960 1965
 Cys Thr Cys Cys Thr Thr Thr Ala Ala Ala Cys Thr Ala Cys Thr
 1970 1975 1980
 Thr Ala Ala Thr Thr Ala Cys Ala Thr Thr Cys Ala Thr Thr Thr
 1985 1990 1995
 Ala Ala Ala Ala Ala Gly Ala Ala Ala Ala Cys Cys Thr Ala Thr
 2000 2005 2010
 Thr Cys Ala Cys Thr Gly Cys Cys Thr Gly Thr Cys Cys Thr Thr
 2015 2020 2025
 Gly Gly Ala Cys Ala Gly Ala Cys Ala Gly Ala Thr Ala Thr Gly
 2030 2035 2040
 Cys Ala Cys Cys Thr Cys Cys Cys Ala Cys Cys Gly Cys Ala Ala
 2045 2050 2055
 Gly Cys Gly Gly Cys Gly Gly Gly Cys Cys Cys Cys Thr Ala Cys
 2060 2065 2070
 Cys Gly Gly Ala Gly Cys Cys Gly Cys Thr Thr Thr Ala Gly Thr
 2075 2080 2085
 Thr Ala Cys Ala Ala Cys Ala Cys Thr Cys Ala Gly Ala Cys Ala
 2090 2095 2100
 Cys Ala Ala Cys Cys Ala Cys Cys Ala Gly Ala Ala Ala Ala Ala
 2105 2110 2115
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 2120 2125 2130
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 2135 2140 2145
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 2150 2155 2160
 Ala Cys Thr Gly Ala Ala Ala Ala Gly Cys Gly Gly Cys Cys Cys
 2165 2170 2175

A-743 PCT.ST25.txt

Cys Gly Cys Cys Cys Cys Gly Gly Thr Cys Cys Gly Ala Ala Gly
 2180 2185 2190
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 2195 2200 2205
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 2240 2245 2250
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 2300 2305 2310
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 2330 2335 2340
 Thr Cys Gly Gly Gly Ala Cys Cys Ala Cys Thr Cys Cys Gly Ala
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 2360 2365 2370
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 2375 2380 2385
 Gly Cys Gly Gly Gly Thr Ala Thr Gly Gly Thr Cys Thr Gly Gly
 2390 2395 2400
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 2405 2410 2415
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 2420 2425 2430

A-743 PCT.ST25.txt

Thr Cys Ala Ala Thr Cys Ala Gly Thr Ala Cys Cys Gly Gly Cys
 2435 2440 2445
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 2450 2455 2460
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 2465 2470 2475
 Thr Thr Thr Cys Ala Thr Ala Thr Ala Thr Gly Ala Ala Ala Cys
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 2495 2500 2505
 Thr Cys Cys Ala Thr Gly Cys Cys Gly Cys Thr Gly Ala Thr Gly
 2510 2515 2520
 Cys Gly Gly Cys Ala Thr Ala Thr Cys Cys Thr Gly Gly Thr Ala
 2525 2530 2535
 Ala Cys Gly Ala Thr Ala Thr Cys Thr Gly Ala Ala Thr Thr Gly
 2540 2545 2550
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 Thr Ala Cys Gly Thr Gly Gly Thr Ala Ala Thr Gly Ala Cys Ala
 2570 2575 2580
 Ala Ala Ala Ala Thr Ala Gly Gly Ala Cys Ala Ala Gly Thr Thr
 2585 2590 2595
 Ala Ala Ala Ala Thr Thr Thr Ala Cys Ala Gly Gly Cys Gly
 2600 2605 2610
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 2615 2620 2625
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 2630 2635 2640
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 2645 2650 2655
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 2675 2680 2685

A-743 PCT.ST25.txt

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 2720 2725 2730
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 2735 2740 2745
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 2750 2755 2760
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 2765 2770 2775
 Gly Cys Gly Thr Cys Gly Cys Thr Thr Gly Gly Thr Cys Gly Gly
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 2825 2830 2835
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 2855 2860 2865
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 2870 2875 2880
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 2885 2890 2895
 Gly Thr Cys Ala Gly Cys Cys Cys Ala Thr Thr Cys Gly Cys Cys
 2900 2905 2910
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 2915 2920 2925
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 2930 2935 2940

A-743 PCT.ST25.txt

Cys Ala Ala Cys Gly Cys Thr Ala Thr Gly Thr Cys Cys Thr Gly
 2945 2950 2955
 Ala Thr Ala Gly Cys Gly Gly Thr Cys Cys Gly Cys Cys Ala Cys
 2960 2965 2970
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 2975 2980 2985
 Gly Thr Cys Gly Ala Thr Gly Ala Ala Thr Cys Cys Ala Gly Ala
 2990 2995 3000
 Ala Ala Ala Gly Cys Gly Gly Cys Cys Ala Thr Thr Thr Thr Cys
 3005 3010 3015
 Cys Ala Cys Cys Ala Thr Gly Ala Thr Ala Thr Thr Cys Gly Gly
 3020 3025 3030
 Cys Ala Ala Gly Cys Ala Gly Gly Cys Ala Thr Cys Gly Cys Cys
 3035 3040 3045
 Ala Thr Gly Ala Gly Thr Cys Ala Cys Gly Ala Cys Gly Ala Gly
 3050 3055 3060
 Ala Thr Cys Cys Thr Cys Gly Cys Cys Gly Thr Cys Gly Gly Gly
 3065 3070 3075
 Cys Ala Thr Gly Cys Gly Cys Gly Cys Cys Thr Thr Gly Ala Gly
 3080 3085 3090
 Cys Cys Thr Gly Gly Cys Gly Ala Ala Cys Ala Gly Thr Thr Cys
 3095 3100 3105
 Gly Gly Cys Thr Gly Gly Cys Gly Cys Gly Ala Gly Cys Cys Cys
 3110 3115 3120
 Cys Thr Gly Ala Thr Gly Cys Thr Cys Thr Thr Cys Gly Thr Cys
 3125 3130 3135
 Cys Ala Gly Ala Thr Cys Ala Thr Cys Cys Thr Gly Ala Thr Cys
 3140 3145 3150
 Gly Ala Cys Ala Ala Gly Ala Cys Cys Gly Gly Cys Thr Thr Cys
 3155 3160 3165
 Cys Ala Thr Cys Cys Gly Ala Gly Thr Ala Cys Gly Thr Gly Cys
 3170 3175 3180
 Thr Cys Gly Cys Thr Cys Gly Ala Thr Gly Cys Gly Ala Thr Gly
 3185 3190 3195

A-743 PCT.ST25.txt

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3215						3220					3225			
Cys	Gly	Gly	Ala	Thr	Cys	Ala	Ala	Gly	Cys	Gly	Thr	Ala	Thr	Gly
3230						3235					3240			
Cys	Ala	Gly	Cys	Cys	Gly	Cys	Cys	Gly	Cys	Ala	Thr	Thr	Gly	Cys
3245						3250					3255			
Ala	Thr	Cys	Ala	Gly	Cys	Cys	Ala	Thr	Gly	Ala	Thr	Gly	Gly	Ala
3260						3265					3270			
Thr	Ala	Cys	Thr	Thr	Thr	Cys	Thr	Cys	Gly	Gly	Cys	Ala	Gly	Gly
3275						3280					3285			
Ala	Gly	Cys	Ala	Ala	Gly	Gly	Thr	Gly	Ala	Gly	Ala	Thr	Gly	Ala
3290						3295					3300			
Cys	Ala	Gly	Gly	Ala	Gly	Ala	Thr	Cys	Cys	Thr	Gly	Cys	Cys	Cys
3305						3310					3315			
Cys	Gly	Gly	Cys	Ala	Cys	Thr	Thr	Cys	Gly	Cys	Cys	Cys	Ala	Ala
3320						3325					3330			
Thr	Ala	Gly	Cys	Ala	Gly	Cys	Cys	Ala	Gly	Thr	Cys	Cys	Cys	Thr
3335						3340					3345			
Thr	Cys	Cys	Cys	Gly	Cys	Thr	Thr	Cys	Ala	Gly	Thr	Gly	Ala	Cys
3350						3355					3360			
Ala	Ala	Cys	Gly	Thr	Cys	Gly	Ala	Gly	Cys	Ala	Cys	Ala	Gly	Cys
3365						3370					3375			
Thr	Gly	Cys	Gly	Cys	Ala	Ala	Gly	Gly	Ala	Ala	Cys	Gly	Cys	Cys
3380						3385					3390			
Cys	Gly	Thr	Cys	Gly	Thr	Gly	Gly	Cys	Cys	Ala	Gly	Cys	Cys	Ala
3395						3400					3405			
Cys	Gly	Ala	Thr	Ala	Gly	Cys	Cys	Gly	Cys	Gly	Cys	Thr	Gly	Cys
3410						3415					3420			
Cys	Thr	Cys	Gly	Thr	Cys	Cys	Thr	Gly	Cys	Ala	Ala	Thr	Thr	Cys
3425						3430					3435			
Ala	Thr	Thr	Cys	Ala	Gly	Gly	Ala	Cys	Ala	Cys	Cys	Gly	Gly	Ala
3440						3445					3450			

A-743 PCT.ST25.txt

Cys Ala Gly Gly Thr Cys Gly Gly Thr Cys Thr Thr Gly Ala Cys
 3455 3460 3465
 Ala Ala Ala Ala Ala Gly Ala Ala Cys Cys Gly Gly Gly Cys Gly
 3470 3475 3480
 Cys Cys Cys Cys Thr Gly Cys Gly Cys Thr Gly Ala Cys Ala Gly
 3485 3490 3495
 Cys Cys Gly Gly Ala Ala Cys Ala Cys Gly Gly Cys Gly Gly Cys
 3500 3505 3510
 Ala Thr Cys Ala Gly Ala Gly Cys Ala Gly Cys Cys Gly Ala Thr
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 Thr Gly Thr Cys Thr Gly Thr Thr Gly Thr Gly Cys Cys Cys Ala
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 Gly Thr Cys Ala Thr Ala Gly Cys Cys Gly Ala Ala Thr Ala Gly
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 3560 3565 3570
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 3575 3580 3585
 Gly Thr Gly Cys Ala Ala Thr Cys Cys Ala Thr Cys Thr Thr Gly
 3590 3595 3600
 Thr Thr Cys Ala Ala Thr Cys Ala Thr Gly Cys Gly Ala Ala Ala
 3605 3610 3615
 Cys Gly Ala Thr Cys Cys Thr Cys Ala Thr Cys Cys Thr Gly Thr
 3620 3625 3630
 Cys Thr Cys Thr Thr Gly Ala Thr Cys Thr Gly Ala Thr Cys Thr
 3635 3640 3645
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 3650 3655 3660
 Thr Cys Ala Gly Ala Thr Cys Cys Thr Thr Gly Gly Cys Gly Gly
 3665 3670 3675
 Cys Ala Ala Gly Ala Ala Ala Gly Cys Cys Ala Thr Cys Cys Ala
 3680 3685 3690
 Gly Thr Thr Thr Ala Cys Thr Thr Thr Gly Cys Ala Gly Gly Gly
 3695 3700 3705

A-743 PCT.ST25.txt

Cys Thr Thr Cys Cys Cys Ala Ala Cys Cys Thr Thr Ala Cys Cys
 3710 3715 3720
 Ala Gly Ala Gly Gly Gly Cys Gly Cys Cys Cys Cys Ala Gly Cys
 3725 3730 3735
 Thr Gly Gly Cys Ala Ala Thr Thr Cys Cys Gly Gly Thr Thr Cys
 3740 3745 3750
 Gly Cys Thr Thr Gly Cys Thr Gly Thr Cys Cys Ala Thr Ala Ala
 3755 3760 3765
 Ala Ala Cys Cys Gly Cys Cys Cys Ala Gly Thr Cys Thr Ala Gly
 3770 3775 3780
 Cys Thr Ala Thr Cys Gly Cys Cys Ala Thr Gly Thr Ala Ala Gly
 3785 3790 3795
 Cys Cys Cys Ala Cys Thr Gly Cys Ala Ala Gly Cys Thr Ala Cys
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 3815 3820 3825
 Gly Cys Thr Thr Gly Cys Gly Thr Thr Thr Thr Cys Cys Cys Thr
 3830 3835 3840
 Thr Gly Thr Cys Cys Ala Gly Ala Thr Ala Gly Cys Cys Cys Ala
 3845 3850 3855
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 3860 3865 3870
 Cys Cys Gly Gly Gly Gly Thr Cys Ala Gly Cys Ala Cys Cys Gly
 3875 3880 3885
 Thr Thr Thr Cys Thr Gly Cys Gly Gly Ala Cys Thr Gly Gly Cys
 3890 3895 3900
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 3905 3910 3915
 Cys Thr Thr Cys Cys Thr Thr Thr Ala Gly Cys Ala Gly Cys Cys
 3920 3925 3930
 Cys Thr Thr Gly Cys Gly Cys Cys Cys Thr Gly Ala Gly Thr Gly
 3935 3940 3945
 Cys Thr Thr Gly Cys Gly Gly Cys Ala Gly Cys Gly Thr Gly Ala
 3950 3955 3960

A-743 PCT.ST25.txt

Ala Gly Cys Thr Ala Cys Ala Thr Ala Thr Ala Thr Gly Thr Gly
3965 3970 3975

Ala Thr Cys Cys Gly Gly Gly Cys Ala Ala Ala Thr Cys Gly Cys
3980 3985 3990

Thr Gly Ala Ala Thr Ala Thr Thr Cys Cys Thr Thr Thr Thr Gly
3995 4000 4005

Thr Cys Thr Cys Cys Gly Ala Cys Cys Ala Thr Cys Ala Gly Gly
4010 4015 4020

Cys Ala Cys Cys Thr Gly Ala Gly Thr Cys Gly Cys Thr Gly Thr
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Cys Thr Thr Thr Thr Cys Gly Thr Gly Ala Cys Ala Thr Thr
4040 4045 4050

Cys Ala Gly Thr Thr Cys Gly Cys Thr Gly Cys Gly Cys Thr Cys
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Ala Cys Gly Gly Cys Thr Cys Thr Gly Gly Cys Ala Gly Thr Gly
4070 4075 4080

Ala Ala Thr Gly Gly Gly Gly Gly Thr Ala Ala Ala Thr Gly Gly
4085 4090 4095

Cys Ala Cys Thr Ala Cys Ala Gly Gly Cys Gly Cys Cys Thr Thr
4100 4105 4110

Thr Thr Ala Thr Gly Gly Ala Thr Thr Cys Ala Thr Gly Cys Ala
4115 4120 4125

Ala Gly Gly Ala Ala Ala Cys Thr Ala Cys Cys Cys Ala Thr Ala
4130 4135 4140

Ala Thr Ala Cys Ala Ala Gly Ala Ala Ala Ala Gly Cys Cys Cys
4145 4150 4155

Gly Thr Cys Ala Cys Gly Gly Gly Cys Thr Thr Cys Thr Cys Ala
4160 4165 4170

Gly Gly Gly Cys Gly Thr Thr Thr Thr Ala Thr Gly Gly Cys Gly
4175 4180 4185

Gly Gly Thr Cys Thr Gly Cys Thr Ala Thr Gly Thr Gly Gly Thr
4190 4195 4200

Gly Cys Thr Ala Thr Cys Thr Gly Ala Cys Thr Thr Thr Thr Thr
4205 4210 4215

A-743 PCT.ST25.txt

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 4220 4225 4230
 Cys Thr Gly Cys Cys Cys Thr Cys Thr Gly Ala Thr Thr Thr Thr
 4235 4240 4245
 Cys Cys Ala Gly Thr Cys Thr Gly Ala Cys Cys Ala Cys Thr Thr
 4250 4255 4260
 Cys Gly Gly Ala Thr Thr Ala Thr Cys Cys Cys Gly Thr Gly Ala
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 Cys Ala Gly Gly Thr Cys Ala Thr Thr Cys Ala Gly Ala Cys Thr
 4280 4285 4290
 Gly Gly Cys Thr Ala Ala Thr Gly Cys Ala Cys Cys Cys Ala Gly
 4295 4300 4305
 Thr Ala Ala Gly Gly Cys Ala Gly Cys Gly Gly Thr Ala Thr Cys
 4310 4315 4320
 Ala Thr Cys Ala Ala Cys Ala Gly Gly Cys Thr Thr Ala Cys Cys
 4325 4330 4335
 Cys Gly Thr Cys Thr Thr Ala Cys Thr Gly Thr Cys Gly Ala Ala
 4340 4345 4350
 Gly Ala Cys Gly Thr Gly Cys Gly Thr Ala Ala Cys Gly Thr Ala
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 Thr Gly Cys Ala Thr Gly Gly Thr Cys Thr Cys Cys Cys Cys Ala
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 4385 4390 4395
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 4400 4405 4410
 Thr Ala Ala Ala Ala Cys Gly Ala Ala Ala Gly Gly Cys Thr Cys
 4415 4420 4425
 Ala Gly Thr Cys Gly Ala Ala Ala Gly Ala Cys Thr Gly Gly Gly
 4430 4435 4440
 Cys Cys Thr Thr Thr Cys Gly Thr Thr Thr Thr Ala Thr Cys Thr
 4445 4450 4455
 Gly Thr Thr Gly Thr Thr Thr Gly Thr Cys Gly Gly Thr Gly Ala
 4460 4465 4470

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Ala Cys Gly Cys Thr Cys Thr Cys Cys Thr Gly Ala Gly Thr Ala
4475 4480 4485

Gly Gly Ala Cys Ala Ala Ala Thr Cys Cys Gly Cys Cys Gly Gly
4490 4495 4500

Gly Ala Gly Cys Gly Gly Ala Thr Thr Thr Gly Ala Ala Cys Gly
4505 4510 4515

Thr Thr Gly Cys Gly Ala Ala Gly Cys Ala Ala Cys Gly Gly Cys
4520 4525 4530

Cys Cys Gly Gly Ala Gly Gly Gly Thr Gly Gly Cys Gly Gly Gly
4535 4540 4545

Cys Ala Gly Gly Ala Cys Gly Cys Cys Cys Gly Cys Cys Ala Thr
4550 4555 4560

Ala Ala Ala Cys Thr Gly Cys Cys Ala Gly Gly Cys Ala Thr Cys
4565 4570 4575

Ala Ala Ala Thr Thr Ala Ala Gly Cys Ala Gly Ala Ala Gly Gly
4580 4585 4590

Cys Cys Ala Thr Cys Cys Thr Gly Ala Cys Gly Gly Ala Thr Gly
4595 4600 4605

Gly Cys Cys Thr Thr Thr Thr Thr Gly Cys Gly Thr Thr Thr Cys
4610 4615 4620

Thr Ala Cys Ala Ala Ala Cys Thr Cys Thr Thr Thr Thr Gly Thr
4625 4630 4635

Thr Thr Ala Thr Thr Thr Thr Thr Cys Thr Ala Ala Ala Thr Ala
4640 4645 4650

Cys Ala Thr Thr Cys Ala Ala Ala Thr Ala Thr Gly Gly Ala Cys
4655 4660 4665

Gly Thr Cys Gly Thr Ala Cys Thr Thr Ala Ala Cys Thr Thr Thr
4670 4675 4680

Thr Ala Ala Ala Gly Thr Ala Thr Gly Gly Gly Cys Ala Ala Thr
4685 4690 4695

Cys Ala Ala Thr Thr Gly Cys Thr Cys Cys Thr Gly Thr Thr Ala
4700 4705 4710

Ala Ala Ala Thr Thr Gly Cys Thr Thr Thr Ala Gly Ala Ala Ala
4715 4720 4725

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Thr Ala Cys Thr Thr Thr Gly Gly Cys Ala Gly Cys Gly Gly Thr
 4730 4735 4740
 Thr Thr Gly Thr Thr Gly Thr Ala Thr Thr Gly Ala Gly Thr Thr
 4745 4750 4755
 Thr Cys Ala Thr Thr Thr Gly Cys Gly Cys Ala Thr Thr Gly Gly
 4760 4765 4770
 Thr Thr Ala Ala Ala Thr Gly Gly Ala Ala Ala Gly Thr Gly Ala
 4775 4780 4785
 Cys Cys Gly Thr Gly Cys Gly Cys Thr Thr Ala Cys Thr Ala Cys
 4790 4795 4800
 Ala Gly Cys Cys Thr Ala Ala Thr Ala Thr Thr Thr Thr Thr Gly
 4805 4810 4815
 Ala Ala Ala Thr Ala Thr Cys Cys Cys Ala Ala Gly Ala Gly Cys
 4820 4825 4830
 Thr Thr Thr Thr Thr Cys Cys Thr Thr Cys Gly Cys Ala Thr Gly
 4835 4840 4845
 Cys Cys Cys Ala Cys Gly Cys Thr Ala Ala Ala Cys Ala Thr Thr
 4850 4855 4860
 Cys Thr Thr Thr Thr Cys Thr Cys Thr Thr Thr Thr Gly Gly
 4865 4870 4875
 Thr Thr Ala Ala Ala Thr Cys Gly Thr Thr Gly Thr Thr Thr Gly
 4880 4885 4890
 Ala Thr Thr Thr Ala Thr Thr Ala Thr Thr Thr Gly Cys Thr Ala
 4895 4900 4905
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 4910 4915 4920
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 4925 4930 4935
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 4940 4945 4950
 Gly Thr Ala Thr Gly Thr Thr Cys Ala Thr Ala Cys Ala Cys Gly
 4955 4960 4965
 Cys Ala Thr Gly Thr Ala Ala Ala Ala Ala Thr Ala Ala Ala Cys
 4970 4975 4980

A-743 PCT.ST25.txt

Thr Ala Thr Cys Thr Ala Thr Ala Thr Ala Gly Thr Thr Gly Thr
 4985 4990 4995
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 Cys Ala Ala Ala Ala Cys Thr Ala Ala Gly Cys Ala Thr Thr Cys
 5015 5020 5025
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 5030 5035 5040
 Cys Ala Gly Thr Ala Thr Gly Ala Ala Thr Ala Gly Gly Gly Ala
 5045 5050 5055
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 5075 5080 5085
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 5090 5095 5100
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 5120 5125 5130
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 5135 5140 5145
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 5180 5185 5190
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 5195 5200 5205
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 5210 5215 5220
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 5225 5230 5235

A-743 PCT.ST25.txt

Thr Ala Gly Gly Gly Thr Ala Ala Thr Thr Ala Thr Cys Cys Ala
 5240 5245 5250
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 5255 5260 5265
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 5270 5275 5280
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 5285 5290 5295
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 5300 5305 5310
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 5315 5320 5325
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 5330 5335 5340
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 5345 5350 5355
 Cys Ala Thr Thr Ala Thr Thr Gly Cys Thr Thr Cys Thr Ala Cys
 5360 5365 5370
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 5375 5380 5385
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 5390 5395 5400
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 5405 5410 5415
 Thr Gly Thr Cys Thr Thr Thr Cys Ala Thr Ala Cys Cys Cys Ala
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 5435 5440 5445
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 5450 5455 5460
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 5465 5470 5475
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 5480 5485 5490

A-743 PCT.ST25.txt

Thr Ala Ala Thr Ala Gly Ala Thr Thr Gly Ala Cys Ala Thr Thr
 5495 5500 5505
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 5510 5515 5520
 Gly Gly Ala Thr Thr Thr Thr Thr Gly Thr Cys Ala Cys Ala Cys
 5525 5530 5535
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 5540 5545 5550
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 5675 5680 5685
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 5690 5695 5700
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 5705 5710 5715
 Thr Cys Thr Gly Gly Gly Ala Cys Gly Gly Thr Gly Cys Thr Gly
 5720 5725 5730
 Thr Ala Ala Cys Ala Ala Ala Thr Gly Thr Gly Ala Ala Cys Cys
 5735 5740 5745

A-743 PCT.ST25.txt

Ala Gly Gly Ala Ala Ala Gly Thr Ala Cys Ala Thr Gly Thr Cys
 5750 5755 5760
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 5765 5770 5775
 Thr Ala Cys Cys Thr Cys Thr Gly Ala Cys Ala Gly Thr Gly Thr
 5780 5785 5790
 Ala Thr Gly Thr Cys Thr Gly Cys Cys Cys Thr Gly Thr Gly Gly
 5795 5800 5805
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 5810 5815 5820
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 5825 5830 5835
 Ala Gly Ala Ala Gly Ala Thr Ala Ala Ala Thr Gly Cys Thr Thr
 5840 5845 5850
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 5855 5860 5865
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 5870 5875 5880
 Cys Cys Thr Gly Gly Thr Gly Gly Cys Cys Gly Thr Gly Gly Thr
 5885 5890 5895
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 5900 5905 5910
 Gly Ala Cys Cys Cys Cys Cys Cys Gly Gly Cys Gly Cys Thr Gly
 5915 5920 5925
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 5930 5935 5940
 Gly Thr Ala Cys Cys Ala Cys Thr Gly Gly Ala Gly Cys Cys Ala
 5945 5950 5955
 Gly Gly Ala Cys Thr Gly Cys Gly Ala Gly Thr Gly Cys Thr Gly
 5960 5965 5970
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 5975 5980 5985
 Gly Thr Gly Cys Gly Cys Gly Cys Cys Gly Gly Gly Cys Cys Thr
 5990 5995 6000

A-743 PCT.ST25.txt

Gly Gly Gly Cys Gly Cys Cys Cys Ala Gly Cys Ala Cys Cys Cys
 6005 6010 6015
 Gly Thr Thr Gly Cys Ala Gly Cys Thr Cys Ala Ala Cys Ala Ala
 6020 6025 6030
 Gly Gly Ala Cys Ala Cys Ala Gly Thr Gly Thr Gly Cys Ala Ala
 6035 6040 6045
 Ala Cys Cys Thr Thr Gly Cys Cys Thr Thr Gly Cys Ala Gly Gly
 6050 6055 6060
 Cys Thr Ala Cys Thr Thr Cys Thr Cys Thr Gly Ala Thr Gly Cys
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 6155 6160 6165
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 6230 6235 6240
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 6245 6250 6255

A-743 PCT.ST25.txt

Thr Cys Cys Gly Gly Ala Ala Cys Thr Cys Cys Thr Gly Gly Gly
 6260 6265 6270
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 6275 6280 6285
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 6290 6295 6300
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 6305 6310 6315
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 6320 6325 6330
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 6350 6355 6360
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 6395 6400 6405
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 6425 6430 6435
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 6455 6460 6465
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 6470 6475 6480
 Cys Ala Cys Cys Gly Thr Cys Cys Thr Gly Cys Ala Cys Cys Ala
 6485 6490 6495
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 6500 6505 6510

A-743 PCT.ST25.txt

Cys Ala Ala Gly Gly Ala Gly Thr Ala Cys Ala Ala Gly Thr Gly
 6515 6520 6525
 Cys Ala Ala Gly Gly Thr Cys Thr Cys Cys Ala Ala Cys Ala Ala
 6530 6535 6540
 Ala Gly Cys Cys Cys Thr Cys Cys Cys Ala Gly Cys Cys Cys Cys
 6545 6550 6555
 Cys Ala Thr Cys Gly Ala Gly Ala Ala Ala Ala Cys Cys Ala Thr
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 Cys Thr Cys Cys Ala Ala Ala Gly Cys Cys Ala Ala Ala Gly Gly
 6575 6580 6585
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 6590 6595 6600
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 6620 6625 6630
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 6635 6640 6645
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 6650 6655 6660
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 6665 6670 6675
 Cys Thr Thr Cys Thr Ala Thr Cys Cys Cys Ala Gly Cys Gly Ala
 6680 6685 6690
 Cys Ala Thr Cys Gly Cys Cys Gly Thr Gly Gly Ala Gly Thr Gly
 6695 6700 6705
 Gly Gly Ala Gly Ala Gly Cys Ala Ala Thr Gly Gly Gly Cys Ala
 6710 6715 6720
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 6740 6745 6750
 Cys Gly Thr Gly Cys Thr Gly Gly Ala Cys Thr Cys Cys Gly Ala
 6755 6760 6765

A-743 PCT.ST25.txt

Cys Gly Gly Cys Thr Cys Cys Thr Thr Cys Thr Thr Cys Cys Thr
 6770 6775 6780
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 6785 6790 6795
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 6800 6805 6810
 Gly Thr Gly Gly Cys Ala Gly Cys Ala Gly Gly Gly Gly Ala Ala
 6815 6820 6825
 Cys Gly Thr Cys Thr Thr Cys Thr Cys Ala Thr Gly Cys Thr Cys
 6830 6835 6840
 Cys Gly Thr Gly Ala Thr Gly Cys Ala Thr Gly Ala Gly Gly Cys
 6845 6850 6855
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 6860 6865 6870
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 6875 6880 6885
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 6890 6895 6900
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 6905 6910 6915
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 6920 6925 6930
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 6935 6940 6945
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 6950 6955 6960
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 6965 6970 6975
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 6980 6985 6990
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 6995 7000 7005
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 7010 7015 7020

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Gly Ala Gly Gly Gly Gly Thr Thr Thr Thr Thr Thr Gly Cys Thr
 7025 7030 7035
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 7040 7045 7050
 Thr Cys Thr Thr Cys Ala Cys Gly Cys Thr Cys Thr Thr Cys Ala
 7055 7060 7065
 Cys Gly Cys Gly Gly Ala Thr Ala Ala Ala Thr Ala Ala Gly Thr
 7070 7075 7080
 Ala Ala Cys Gly Ala Thr Cys Cys Gly Gly Thr Cys Cys Ala Gly
 7085 7090 7095
 Thr Ala Ala Thr Gly Ala Cys Cys Thr Cys Ala Gly Ala Ala Cys
 7100 7105 7110
 Thr Cys Cys Ala Thr Cys Thr Gly Gly Ala Thr Thr Thr Gly Thr
 7115 7120 7125
 Thr Cys Ala Gly Ala Ala Cys Gly Cys Thr Cys Gly Gly Thr Thr
 7130 7135 7140
 Gly Cys Cys Gly Cys Cys Gly Gly Gly Cys Gly Thr Thr Thr Thr
 7145 7150 7155
 Thr Thr Ala Thr Thr Gly Gly Thr Gly Ala Gly Ala Ala Thr Cys
 7160 7165 7170
 Gly Cys Ala Gly Cys Ala Ala Cys Thr Thr Gly Thr Cys Gly Cys
 7175 7180 7185
 Gly Cys Cys Ala Ala Thr Cys Gly Ala Gly Cys Cys Ala Thr Gly
 7190 7195 7200
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 7205 7210 7215
 Cys Cys Cys Cys Ala Thr Thr Cys Ala Ala Gly Ala Ala Cys Ala
 7220 7225 7230
 Gly Cys Ala Ala Gly Cys Ala Gly Cys Ala Thr Thr Gly Ala Gly
 7235 7240 7245
 Ala Ala Cys Thr Thr Thr Gly Gly Ala Ala Thr Cys Cys Ala Gly
 7250 7255 7260
 Thr Cys Cys Cys Thr Cys Thr Thr Cys Cys Ala Cys Cys Thr Gly
 7265 7270 7275

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Cys Thr Gly Ala Cys Cys Gly
7280 7285

<210> 29
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 Modulating Domains
<400> 29

Pro Gly Thr Cys Phe Pro Phe Pro Trp Glu Cys Thr His Ala
1 5 10

<210> 30
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 Modulating Domains
<400> 30

Trp Gly Ala Cys Trp Pro Phe Pro Trp Glu Cys Phe Lys Glu
1 5 10

<210> 31
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 Modulating Domains
<400> 31

Val Pro Phe Cys Asp Leu Leu Thr Lys His Cys Phe Glu Ala
1 5 10

<210> 32
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 Modulating Domains
<400> 32

Gly Ser Arg Cys Lys Tyr Lys Trp Asp Val Leu Thr Lys Gln Cys Phe
1 5 10 15

His His

<210> 33
<211> 18
<212> PRT

A-743 PCT.ST25.txt

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 Modulating Domains

<400> 33

Leu	Pro	Gly	Cys	Lys	Trp	Asp	Leu	Leu	Ile	Lys	Gln	Trp	Val	Cys	Asp
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Pro Leu

<210> 34

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 Modulating Domains

<400> 34

Ser	Ala	Asp	Cys	Tyr	Phe	Asp	Ile	Leu	Thr	Lys	Ser	Asp	Val	Cys	Thr
1				5					10					15	

Ser Ser

<210> 35

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 Modulating Domains

<400> 35

Ser	Asp	Asp	Cys	Met	Tyr	Asp	Gln	Leu	Thr	Arg	Met	Phe	Ile	Cys	Ser
1				5					10					15	

Asn Leu

<210> 36

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 Modulating Domains

<400> 36

Asp	Leu	Asn	Cys	Lys	Tyr	Asp	Glu	Leu	Thr	Tyr	Lys	Glu	Trp	Cys	Gln
1				5					10					15	

Phe Asn

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<210> 37
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 Modulating Domains

<400> 37

Phe His Asp Cys Lys Tyr Asp Leu Leu Thr Arg Gln Met Val Cys His
 1 5 10 15

Gly Leu

<210> 38
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 Modulating Domains

<400> 38

Arg Asn His Cys Phe Trp Asp His Leu Leu Lys Gln Asp Ile Cys Pro
 1 5 10 15

Ser Pro

<210> 39
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 Modulating Domains

<400> 39

Ala Asn Gln Cys Trp Trp Asp Ser Leu Thr Lys Lys Asn Val Cys Glu
 1 5 10 15

Phe Phe

<210> 40
 <211> 8
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Polyglycine linkers

<400> 40
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8

<210> 41

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<211> 8
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Polyglycine linkers

<220>
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 <222> (4)..(4)
 <223> N is asparagine

<400> 41
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8

<210> 42
 <211> 8
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Polyglycine linkers

<400> 42
 gggcgggg

8

<210> 43
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Polyglycine linkers

<400> 43

Gly Pro Asn Gly Gly
 1 5

<210> 44
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide Bond

<220>
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 <223> Xaa = a peptide bond
 Fc domain attached at Position 19 to C-terminus

<400> 44

Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp
 1 5 10 15

Pro Leu Xaa

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<210> 45
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<400> 45

Xaa Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
 1 5 10 15

Asp Pro Leu

<210> 46
 <211> 38
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (38)..(38)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 38 to C-terminus

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa = a peptide bond

<400> 46

Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys Asp
 1 5 10 15

Pro Leu Xaa Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp
 20 25 30

Val Cys Asp Pro Leu Xaa
 35

<210> 47
 <211> 38
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

A-743 PCT.ST25.txt

<220>
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 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<220>
 <221> misc_feature
 <222> (20)..(20)
 <223> Xaa = a peptide bond

<400> 47

Xaa Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
 1 5 10 15

Asp Pro Leu Xaa Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln
 20 25 30

Trp Val Cys Asp Pro Leu
 35

<210> 48
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 19 to C-terminus

<400> 48

Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val Cys Thr
 1 5 10 15

Ser Ser Xaa

<210> 49
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<400> 49

A-743 PCT.ST25.txt

Xaa Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val Cys
 1 5 10 15

Thr Ser Ser

<210> 50
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (36)..(36)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 36 to C-terminus

<220>
 <221> misc_feature
 <222> (18)..(18)
 <223> Xaa = a peptide bond

<400> 50

Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val Thr Ser
 1 5 10 15

Ser Xaa Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val
 20 25 30

Thr Ser Ser Xaa
 35

<210> 51
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa = a peptide bond

<400> 51

Xaa Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val Thr
 Page 49

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1 5 10 15

Ser Ser Xaa Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp
 20 25 30

Val Thr Ser Ser
 35

<210> 52
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 19 to C-terminus

<400> 52

Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys His
 1 5 10 15

Gly Leu Xaa

<210> 53
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<400> 53

Xaa Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys
 1 5 10 15

His Gly Leu

<210> 54
 <211> 38
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

A-743 PCT.ST25.txt

<220>
 <221> misc_feature
 <222> (19)..(19)
 <223> Xaa = a peptide bond

<220>
 <221> misc_feature
 <222> (38)..(38)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 38 to C-terminus

<400> 54

Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys His
 1 5 10 15

Gly Leu Xaa Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp
 20 25 30

Val Cys His Gly Leu Xaa
 35

<210> 55
 <211> 38
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide bond

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa = a peptide bond
 Fc domain attached at Position 1 to N-terminus

<220>
 <221> misc_feature
 <222> (20)..(20)
 <223> Xaa = a peptide bond

<400> 55

Xaa Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys
 1 5 10 15

His Gly Leu Xaa Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln
 20 25 30

Trp Val Cys His Gly Leu
 35

<210> 56
 <211> 25
 <212> DNA
 <213> Artificial Sequence

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<220>

<223> Oligonucleotide

<400> 56

cggcgcaact atcgggtatca agctg

25

<210> 57

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide

<400> 57

catgtaccgt aacactgagt ttcgtc

26

<210> 58

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Consensus peptide

<400> 58

Phe	His	Asp	Cys	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Val	Cys	His
1				5					10					15	

Gly Leu

<210> 59

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred linker sequence

<400> 59

Gly	Ser	Gly	Ser	Ala	Thr	Gly	Gly	Ser	Gly	Ser	Thr	Ala	Ser	Ser	Gly
1				5					10					15	

Ser	Gly	Ser	Ala	Thr	His	Met
					20	

<210> 60

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 60

Asn	Gln	Thr	Leu	Trp	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Phe	Ile	Thr
1				5					10					15	

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Tyr Met

<210> 61
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 61

Pro Val Tyr Gln Gly Trp Trp Asp Thr Leu Thr Lys Leu Tyr Ile Trp
 1 5 10 15

Asp Gly

<210> 62
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 62

Trp Leu Asp Gly Gly Trp Arg Asp Pro Leu Ile Lys Arg Ser Val Gln
 1 5 10 15

Leu Gly

<210> 63
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 63

Gly His Gln Gln Phe Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Gln
 1 5 10 15

Ser Asn

<210> 64
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 64

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Gln Arg Val Gly Gln Phe Trp Asp Val Leu Thr Lys Met Phe Ile Thr
 1 5 10 15

Gly Ser

<210> 65
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 65

Gln Ala Gln Gly Trp Ser Tyr Asp Ala Leu Ile Lys Thr Trp Ile Arg
 1 5 10 15

Trp Pro

<210> 66
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 66

Gly Trp Met His Trp Lys Trp Asp Pro Leu Thr Lys Gln Ala Leu Pro
 1 5 10 15

Trp Met

<210> 67
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 67

Gly His Pro Thr Tyr Lys Trp Asp Leu Leu Thr Lys Gln Trp Ile Leu
 1 5 10 15

Gln Met

<210> 68
 <211> 18
 <212> PRT
 <213> Artificial Sequence

A-743 PCT.ST25.txt

<220>

<223> Preferred TALL-1 modulating domains

<400> 68

Trp Asn Asn Trp Ser Leu Trp Asp Pro Leu Thr Lys Leu Trp Leu Gln
1 5 10 15

Gln Asn

<210> 69

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 69

Trp Gln Trp Gly Trp Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Gln
1 5 10 15

Gln Gln

<210> 70

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 70

Gly Gln Met Gly Trp Arg Trp Asp Pro Leu Thr Lys Met Trp Leu Gly
1 5 10 15

Thr Ser

<210> 71

<211> 62

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 71

tatgccgggt acttgtttcc cgttcccgtg ggaatgcact cacgctgggtg gaggcgggtgg 60

99

<210> 72

<211> 64

<212> DNA

<213> Artificial Sequence

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<220>
<223> Oligonucleotides

<400> 72
tcgacccac cgcctcctgg agcgtgagtg cattcccacg ggaagccgaa acaagtaccc 60
ggca 64

<210> 73
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 73
tatgtggggg gcttggtggc cgttcccgtg ggaatgtttc aaagaagggtg gaggcgggtgg 60
gg 62

<210> 74
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 74
tcgacccac cgcctccacc ttctttgaaa cattcccacg ggaacggcca acaagcaccc 60
caca 64

<210> 75
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 75
tatggttcg ttctgtgacc tgctgactaa acactgtttc gaagctggtg gaggcgggtgg 60
gg 62

<210> 76
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 76
tcgacccac cgcctccacc agcttcgaaa cagtgttttag tcagcaggtc acagaacgga 60
acca 64

<210> 77
<211> 74
<212> DNA

A-743 PCT.ST25.txt

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 77

tatgggttct cgttgtaaata acaaattggga cgttctgact aaacagtgtt tccaccacgg 60

tggaggcggt gggg 74

<210> 78

<211> 76

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 78

tcgacccac cgcctccacc gtggtggaaa cactgttttag tcagaacgct ccatgttat 60

ttacaacgag aaccca 76

<210> 79

<211> 74

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 79

tatgtctgccg ggttgtaaata gggacctgct gatcaaacag tgggtttgtg acccgctggg 60

tggaggcggt gggg 74

<210> 80

<211> 76

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 80

tcgacccac cgcctccacc cagcgggtca caaacccact gtttgatcag cagggtcccat 60

ttacaacccg gcagca 76

<210> 81

<211> 74

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotides

<400> 81

tatgtctgct gactgttact tcgacatcct gactaaatct gacgtttgta cttcttctgg 60

tggaggcggt gggg 74

<210> 82

A-743 PCT.ST25.txt

<211> 76
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 82
tcgacccac cgcctccacc agaagaagta caaacgtcag atttagtcag gatgtcgaag 60
taacagtcag cagaca 76

<210> 83
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 83
tatgtctgac gactgtatgt acgaccagct gactcgtatg ttcattctgtt ctaacctggg 60
tggaggcggt gggg 74

<210> 84
<211> 76
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 84
tcgacccac cgcctccacc caggtagaa cagatgaaca tacgagtcag ctggctgtac 60
atacagtcgt cagaca 76

<210> 85
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 85
tatggacctg aactgtaaat acgacgaact gacttacaaa gaatggtgtc agttcaacgg 60
tggaggcggt gggg 74

<210> 86
<211> 76
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 86
tcgacccac cgcctccacc gttgaactga caccattctt tgtaagtcag ttcgtcgtat 60
ttacagttca ggtcca 76

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<210> 87
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 87
tatgttccac gactgtaaat acgacctgct gactcgtcag atggtttgtc acggtctggg 60
tggaggcggt gggg 74

<210> 88
<211> 76
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 88
tcgacccac cgctccacc cagaccgtga caaaccatct gacgagtcag caggctcgat 60
ttacagtcgt ggaaca 76

<210> 89
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 89
tatgcgtaac cactgtttct gggaccacct gctgaaacag gacatctgtc cgtctccggg 60
tggaggcggt gggg 74

<210> 90
<211> 76
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 90
tcgacccac cgctccacc cggagacgga cagatgtcct gtttcagcag gtggtcccag 60
aaacagtggc tacgca 76

<210> 91
<211> 74
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotides

<400> 91
tatggctaac cagtgttggc gggactctct gctgaaaaaa aacgtttgtg aattcttcgg 60

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tggaggcggt gggg

74

<210> 92
 <211> 76
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotides

<400> 92
 tcgacccac cgcctccacc gaagaattca caaacgtttt ttttcagcag agagtccac 60
 caacactggt tagcca 76

<210> 93
 <211> 74
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotides

<400> 93
 tatgttccac gactgcaaat gggacctgct gaccaaacag tgggtttgcc acggtctggg 60
 tggaggcggt gggg 74

<210> 94
 <211> 76
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotides

<400> 94
 tcgacccac cgcctccacc cagaccgtgg caaacccact gtttggtcag caggtcccat 60
 ttgcagtcgt ggaaca 76

<210> 95
 <211> 141
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pAMG21-RANK-Fc vector

<400> 95
 ctaattccgc tctcacctac caaacaatgc cccctgcaa aaaataaatt catataaaaa 60
 acatacagat aaccatctgc ggtgataaat tatctctggc ggtgttgaca taaataccac 120
 tggcggtgat actgagcaca t 141

<210> 96
 <211> 55
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> pAMG21-RANK-Fc vector

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<400> 96
cgatttgatt ctagaaggag gaataacata tggtaacgc gttggaattc ggtac 55

<210> 97
<211> 1546
<212> DNA
<213> Artificial Sequence

<220>
<223> pAMG21

<400> 97
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cgaaaggctc agtcgaaaga ctgggccttt cgttttatct gttgtttgtc ggtgaacgct 120
ctcctgagta ggacaaatcc gccgggagcg gatttgaacg ttgcgaagca acggcccgga 180
gggtggcggg caggacgccc gccataaact gccaggcatc aaattaagca gaaggccatc 240
ctgacgggatg gcctttttgc gtttctacaa actcttttgc ttatttttct aaatacattc 300
aaatatggac gtcgtactta acttttaaag tatgggcaat caattgctcc tgttaaaatt 360
gcttttagaaa tactttggca gcggtttgtt gtattgagtt tcatttgccg atttggttaa 420
tggaagtgta ccgtgcgctt actacagcct aatatttttg aaatatccca agagcttttt 480
ccttcgcatg cccacgctaa acattctttt tctcttttgg ttaaatcggt gtttgattta 540
ttatttgcta tatttttttt tcgataatta tcaactagag aaggaacaat taatggtatg 600
ttcatacacg catgtaaaaa taaactatct atagattgtt ctttctctga atgtgcaaaa 660
ctaagcattc cgaagccatt attagcagta tgaataggga aactaaacc agtgataaga 720
cctgatgatt tcgcttcttt aattacattt ggagattttt tatttacagc attgttttca 780
aatatattcc aattaatcgg tgaatgattg gagttagaat aatctactat aggatcatat 840
tttattaaat tagcgtcatc ataatttgc ctccattttt tagggtaatt atccagaatt 900
gaaatatcag atttaaccat agaatgagga taaatgatcg cgagtaaata atattcacia 960
tgtaccattt tagtcatatc agataagcat tgattaatat cattattgct tctacaggct 1020
ttaattttat taattattct gtaagtgtcg tcggcattta tgtctttcat acccatctct 1080
ttatccttac ctattgtttg tcgcaagttt tgcgtgttat atatcattaa aacggtaata 1140
gattgacatt tgattctaatt aaattggatt tttgtcacac tattatatcg cttgaaatac 1200
aattgtttta cataagtacc tgtaggatcg tacaggttta cgcaagaaaa tggtttggtta 1260
tagtcgatta atcgatttga ttctagattt gttttaacta attaaaggag gaataacata 1320
tggtaacgc gttggaattc gagctcacta gtgtcgacct gcagggtacc atggaagctt 1380
actcgaggat ccgcggaaaag aagaagaaga agaagaaagc ccgaaaggaa gctgagttgg 1440
ctgctgccac cgctgagcaa taactagcat aacccttgg ggcctctaaa cgggtcttga 1500
ggggtttttt gctgaaagga ggaaccgctc ttcacgctct tcacgc 1546

<210> 98

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<211> 872
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> GM221

<400> 98
 ttatttttcgt gcgggccgcac cattatcacc gccagaggta aactagtcaa cacgcacggt 60
 gttagatatt tatcccttgc ggtgatagat tgagcacatc gatttgattc tagaaggagg 120
 gataatatat gagcacaaaa aagaaacat taacacaaga gcagcttgag gacgcacgtc 180
 gccttaaagc aatttatgaa aaaaagaaaa atgaacttgg cttatcccag gaatctgtcg 240
 cagacaagat ggggatgggg cagtcaggcg ttggtgcttt atttaatggc atcaatgcat 300
 taaatgctta taacgccgca ttgcttacia aaattctcaa agttagcgtt gaagaattta 360
 gcccttcaat cgccagagaa tctacgagat gtatgaagcg gttagtatgc agccgtcact 420
 tagaagttag tatgagtacc ctgttttttc tcatgttcag gcagggatgt tctcacctaa 480
 gcttagaacc ttaccaaaag gtgatgcgga gagatgggta agcacaacca aaaaagccag 540
 tgattctgca ttctggcttg aggttgaagg taattccatg accgcaccaa caggctccaa 600
 gccagcttt cctgacggaa tgtaattct cgttgaccct gagcaggctg ttgagccagg 660
 tgatttctgc atagccagac ttgggggtga tgagtttacc ttcaagaaac tgatcaggga 720
 tagcggtcag gtgtttttac aaccactaaa cccacagtac ccaatgatcc catgcaatga 780
 gagttgttcc gttgtgggga aagttatcgc tagtcagtgg cctgaagaga cgtttggctg 840
 atagactagt ggatccacta gtgtttctgc cc 872

<210> 99
 <211> 1197
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> GM221

<400> 99
 ggcggaaacc gacgtccatc gaatggtgca aaacctttcg cggatatggca tgatagcgcc 60
 cggaagagag tcaattcagg gtggtgaatg tgaaaccagt aacgttatac gatgtcgag 120
 agtatgccgg tgtctcttat cagaccgttt cccgcgtggg gaaccaggcc agccacgttt 180
 ctgcgaaaac gcgggaaaaa gtcgaagcgg cgatggcgga gctgaattac attcccaacc 240
 gcgtggcaca acaactggcg ggcaaacagt cgctcctgat tggcgttgcc acctccagtc 300
 tggccctgca cgcgccgtcg caaattgtcg cggcgattaa atctcgcgcc gatcaactgg 360
 gtgccagcgt ggtggtgtcg atggtagaac gaagcggcgt cgaagcctgt aaagcggcgg 420
 tgcacaatct tctcgcgcaa cgcgtcagtg ggctgatcat taactatccg ctggatgacc 480
 aggatgcat tgctgtggaa gctgcctgca ctaatgttcc ggcgttattt cttgatgtct 540
 ctgaccagac acccatcaac agtattattt tctcccatga agacggtacg cgactgggcg 600

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tggagcatct ggtcgcatg ggtcaccagc aaatcgcgct gttagcgggc ccattaagtt 660
 ctgtctcggc gcgtctcggt ctggctggct ggcataaata tctcactcgc aatcaaattc 720
 agccgatagc ggaacgggaa ggcgactgga gtgccatgtc cggttttcaa caaaccatgc 780
 aaatgctgaa tgagggcatc gttcccactg cgatgctggt tgccaacgat cagatggcgc 840
 tgggcgcaat gcgcgccatt accgagtccg ggctgcgcgt tggcgcgat atctcggtag 900
 tgggatacga cgataccgaa gacagctcat gttatatccc gccgttaacc accatcaaac 960
 aggattttcg cctgctgggg caaaccagcg tggaccgctt gctgcaactc tctcagggcc 1020
 aggcggtgaa gggcaatcag ctgttgcccg tctcactggt gaaaagaaaa accaccctgg 1080
 cgcccaatac gcaaaccgcc tctccccgcg cgttggccga ttcattaatg cagctggcac 1140
 gacaggtttc ccgactggaa agcggacagt aaggtacatc aggatccagg cacagga 1197

<210> 100
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulators of TALL-1

<220>
 <221> misc_feature
 <222> (1, 2, 3, 13)..(14)
 <223> Xaa (Pos1,2,3,13,14) are each independently absent or amino acid residues;

<220>
 <221> misc_feature
 <222> (6)..(6)
 <223> Xaa (Pos6) is an amino acid residue; Xaa (Pos9) is a basic or hydrophobic residue;

<220>
 <221> misc_feature
 <222> (12)..(12)
 <223> Xaa (Pos12) is a neutral hydrophobic residue.

<400> 100

Xaa Xaa Xaa Cys Asp Xaa Leu Thr Xaa Xaa Cys Xaa Xaa Xaa
 1 5 10

<210> 101
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulators of TALL-1

<220>
 <221> misc_feature
 <222> (1, 2, 3, 12 and)..(13)
 <223> Xaa (Pos1,2,3,12,13) are each independently absent or amino acid

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residues;

<220>
 <221> misc_feature
 <222> (5 and)..(8)
 <223> Xaa (Pos5,8) is a neutral hydrophobic residue; Xaa (Pos10) is an acidic residue;

<220>
 <221> misc_feature
 <222> (14)..(14)
 <223> Xaa (Pos14) is absent or is an amino acid residue.

<400> 101

Xaa Xaa Xaa Cys Xaa Pro Phe Xaa Trp Xaa Cys Xaa Xaa Xaa
 1 5 10

<210> 102
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (1, 2, 3, 12, 13 and)..(14)
 <223> Xaa (Pos1,2,3,12,13,14) are each independently absent or amino acid residues;

<220>
 <221> misc_feature
 <222> (6 and)..(7)
 <223> Xaa (Pos6,7) is a hydrophobic residue;

<220>
 <221> misc_feature
 <222> (10)..(10)
 <223> Xaa (Pos10) is an acidic or polar hydrophobic residue.

<400> 102

Xaa Xaa Xaa Xaa Trp Xaa Xaa Trp Gly Xaa Xaa Xaa Xaa Xaa
 1 5 10

<210> 103
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (1)..(1)
 <223> Xaa (Pos1) is absent or is an amino acid residue;

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<220>
<221> misc_feature
<222> (2 and)..(14)
<223> Xaa (Pos2,14) is a neutral hydrophobic residue;

<220>
<221> misc_feature
<222> (3 and)..(10)
<223> Xaa (Pos3,10) is an amino acid residue;

<220>
<221> misc_feature
<222> (5, 6, 7, 8, 12 and)..(13)
<223> Xaa (Pos5,6,7,8,12,13) are each independently amino acid residues
;

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa (Pos9) is an acidic residue.

<400> 103

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
1 5 10

<210> 104
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Modulator of TALL-1

<220>
<221> misc_feature
<222> (1, 2, 12, 13, 16, 17 and)..(18)
<223> Xaa (Pos1,2,12,13,16,17,18) are each independently absent or amino acid residues;

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa (Pos3) is an acidic or amide residue;

<220>
<221> misc_feature
<222> (5 and)..(8)
<223> Xaa (Pos5,8) is an amino acid residue;

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa (Pos6) is an aromatic residue;

<220>
<221> misc_feature
<222> (11)..(11)

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<223> Xaa (Pos11) is a basic residue;

<220>

<221> misc_feature

<222> (14)..(14)

<223> Xaa (Pos14) is a neutral hydrophobic residue.

<400> 104

Xaa	Xaa	Xaa	Cys	Xaa	Xaa	Asp	Xaa	Leu	Thr	Xaa	Xaa	Xaa	Xaa	Cys	Xaa
1			5					10						15	

Xaa Xaa

<210> 105

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Modulator of TALL-1

<220>

<221> misc_feature

<222> (1, 2 and)..(3)

<223> Xaa (Pos1,2,3) are each independently absent or amino acid residues;

<220>

<221> misc_feature

<222> (5, 7, 14 and)..(16)

<223> Xaa (Pos5,7,14,16) is an amino acid residue;

<220>

<221> misc_feature

<222> (10)..(10)

<223> Xaa (Pos10) is a basic residue;

<220>

<221> misc_feature

<222> (11 and)..(12)

<223> Xaa (Pos11,12) are each independently amino acid residues;

<220>

<221> misc_feature

<222> (13 and)..(17)

<223> Xaa (Pos13,17) is a neutral hydrophobic residue;

<220>

<221> misc_feature

<222> (18)..(18)

<223> Xaa (Pos18) is an amino acid residue or is absent.

<400> 105

Xaa Xaa Xaa Cys Xaa Asp Xaa Leu Thr Xaa Xaa Xaa Xaa Xaa Cys Xaa

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1

5

10

15

Xaa Xaa

<210> 106
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (1, 2, 3, 16, 17 and)..(18)
 <223> Xaa (Pos1,2,3,16,17,18) are each independently absent or amino acid residues;

<220>
 <221> misc_feature
 <222> (5, 6, 7, 10, 13 and)..(14)
 <223> Xaa (Pos5,6,7,10,13,14) are each independently amino acid residues.

<400> 106

Xaa Xaa Xaa Cys Xaa Xaa Xaa Trp Asp Xaa Leu Thr Xaa Xaa Cys Xaa
 1 5 10 15

Xaa Xaa

<210> 107
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (1,2,3,15,16,17)..(18)
 <223> Xaa (Pos1,2,3,15,16,17,18) are each independently absent or amino acid residues;

<220>
 <221> misc_feature
 <222> (5, 6, 7, 9 and)..(13)
 <223> Xaa (Pos 5,6,7,9 13) are each independently amino acid residues;

<220>
 <221> misc_feature
 <222> (11)..(11)
 <223> Xaa (Pos 11) is T or I; and

<400> 107

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Xaa Xaa Xaa Cys Xaa Xaa Xaa Asp Xaa Leu Xaa Lys Xaa Cys Xaa Xaa
 1 5 10 15

Xaa Xaa

<210> 108
 <211> 4
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (2)..(2)
 <223> X at (Pos 2) is an amino acid residue

<220>
 <221> misc_feature
 <222> (4)..(4)
 <223> X at (Pos 4) is threonyl or isoleucyl

<400> 108

Asp Xaa Leu Xaa
 1

<210> 109
 <211> 14
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modulator of TALL-1

<220>
 <221> misc_feature
 <222> (1, 2 and)..(3)
 <223> X at (Pos 1, 2, 3) are absent or are amino acid residues (with on
 e of X1, X2, and X3 preferred to be C when one of X12,
 X13, and
 d X14 is C);

<220>
 <221> misc_feature
 <222> (5)..(5)
 <223> X at (Pos 5) is W, Y, or F (W preferred);

<220>
 <221> misc_feature
 <222> (7)..(7)
 <223> X at (Pos 7) is an amino acid residue (L preferred);

<220>
 <221> misc_feature
 <222> (9)..(9)
 <223> X at (Pos 9) is T or I (T preferred);

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<220>
<221> misc_feature
<222> (10)..(10)
<223> X at (Pos 10) is K, R, or H ( K preferred).

<220>
<221> misc_feature
<222> (12)..(12)
<223> X at (Pos 12) is C, a neutral hydrophobic residue, or a basic res
       idue (W, C, or R                      preferred);

<220>
<221> misc_feature
<222> (13)..(13)
<223> X at (Post 13) is C, a neutral hydrophobic residue or is absent
       (V preferred);

<220>
<221> misc_feature
<222> (14)..(14)
<223> X at (Pos 14) is any amino acid residue or is absent.

<400> 109

Xaa Xaa Xaa Lys Xaa Asp Xaa Leu Xaa Xaa Gln Xaa Xaa Xaa
1          5          10

<210> 110
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Modulator of TALL-1

<400> 110

Pro Phe Pro Trp Glu
1          5

<210> 111
<211> 248
<212> PRT
<213> Artificial Sequence

<220>
<223> TALL-1 inhibitory peptibodies

<400> 111

Met Pro Gly Thr Cys Phe Pro Phe Pro Trp Glu Cys Thr His Ala Gly
1          5          10          15

Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
20          25          30

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
35          40          45

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Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 50 55 60
 Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 65 70 75 80
 Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 85 90 95
 Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 100 105 110
 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 115 120 125
 Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 130 135 140
 Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 145 150 155 160
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 165 170 175
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 180 185 190
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 195 200 205
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 210 215 220
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 225 230 235 240
 Ser Leu Ser Leu Ser Pro Gly Lys
 245

<210> 112

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 112

Met Trp Gly Ala Cys Trp Pro Phe Pro Trp Glu Cys Phe Lys Glu Gly
 1 5 10 15

Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
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20

25

30

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 35 40 45

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 50 55 60

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 65 70 75 80

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 85 90 95

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 100 105 110

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 115 120 125

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 130 135 140

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 145 150 155 160

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 165 170 175

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 180 185 190

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 195 200 205

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 210 215 220

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 225 230 235 240

Ser Leu Ser Leu Ser Pro Gly Lys
 245

<210> 113

<211> 248

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 113

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Met Val Pro Phe Cys Asp Leu Leu Thr Lys His Cys Phe Glu Ala Gly
1 5 10 15

Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
20 25 30

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
35 40 45

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
50 55 60

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
65 70 75 80

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
85 90 95

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
100 105 110

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
115 120 125

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
130 135 140

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
145 150 155 160

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
165 170 175

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
180 185 190

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
195 200 205

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
210 215 220

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
225 230 235 240

Ser Leu Ser Leu Ser Pro Gly Lys
245

<210> 114

<211> 252

<212> PRT

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<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 114

Met Gly Ser Arg Cys Lys Tyr Lys Trp Asp Val Leu Thr Lys Gln Cys
 1 5 10 15

Phe His His Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

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250

245

<210> 115
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> TALL-1 inhibitory peptibodies

<400> 115

Met Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
1 5 10 15

Asp Pro Leu Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
210 215 220

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Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 116

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 116

Met Ser Ala Asp Cys Tyr Phe Asp Ile Leu Thr Lys Ser Asp Val Cys
 1 5 10 15

Thr Ser Ser Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
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195
200
205
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Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
210 215 220
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
225 230 235 240
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
245 250
<210> 117
<211> 252
<212> PRT
<213> Artificial Sequence
<220>
<223> TALL-1 inhibitory peptibodies
<400> 117
Met Ser Asp Asp Cys Met Tyr Asp Gln Leu Thr Arg Met Phe Ile Cys
1 5 10 15
Ser Asn Leu Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
20 25 30
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
35 40 45
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
50 55 60
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
65 70 75 80
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
85 90 95
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
100 105 110
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
115 120 125
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
130 135 140
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
145 150 155 160
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
165 170 175

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Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 118

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 118

Met Asp Leu Asn Cys Lys Tyr Asp Glu Leu Thr Tyr Lys Glu Trp Cys
 1 5 10 15

Gln Phe Asn Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
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145 150 155 160
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 165 170 175
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190
 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 195 200 205
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 210 215 220
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

 <210> 119
 <211> 252
 <212> PRT
 <213> Artificial Sequence

 <220>
 <223> TALL-1 inhibitory peptibodies

 <400> 119
 Met Phe His Asp Cys Lys Tyr Asp Leu Leu Thr Arg Gln Met Val Cys
 1 5 10 15
 His Gly Leu Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30
 Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 85 90 95
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 100 105 110
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 115 120 125

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Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 120

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 120

Met Arg Asn His Cys Phe Trp Asp His Leu Leu Lys Gln Asp Ile Cys
 1 5 10 15

Pro Ser Pro Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
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100

105

110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
 130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 121

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 121

Met Ala Asn Gln Cys Trp Trp Asp Ser Leu Thr Lys Lys Asn Val Cys
 1 5 10 15

Glu Phe Phe Gly Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
 20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
 35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 50 55 60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 65 70 75 80

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Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
245 250

<210> 122

<211> 252

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 122

Met Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys
1 5 10 15

His Gly Leu Gly Gly Gly Gly Val Asp Lys Thr His Thr Cys Pro
20 25 30

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
35 40 45

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
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50

55

60

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
65 70 75 80

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
85 90 95

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
100 105 110

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
115 120 125

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
130 135 140

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
145 150 155 160

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
165 170 175

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
180 185 190

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
195 200 205

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
210 215 220

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
225 230 235 240

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
245 250

$\langle 210 \rangle$ 123

<211> 293

<212> PRT

<213> Artificial Sequence

<220>

<223> TALL-1 inhibitory peptibodies

<400> 123

Met Leu Pro Gly Cys Lys Trp Asp Leu Leu Ile Lys Gln Trp Val Cys
1 5 10 15

Asp Pro Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala
20 25 30

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Ser Ser Gly Ser Gly Ser Ala Thr His Met Leu Pro Gly Cys Lys Trp
 35 40 45
 Asp Leu Leu Ile Lys Gln Trp Val Cys Asp Pro Leu Gly Gly Gly Gly
 50 55 60
 Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 65 70 75 80
 Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 85 90 95
 Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 100 105 110
 Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 115 120 125
 Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 130 135 140
 Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 145 150 155 160
 Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 165 170 175
 Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 180 185 190
 Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 195 200 205
 Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 210 215 220
 Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 225 230 235 240
 Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 245 250 255
 Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 260 265 270
 Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 275 280 285
 Leu Ser Pro Gly Lys
 290

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<210> 124
 <211> 293
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> TALL-1 inhibitory peptibodies

<400> 124

Met Phe His Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Cys
 1 5 10 15

His Gly Leu Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala
 20 25 30

Ser Ser Gly Ser Gly Ser Ala Thr His Met Phe His Asp Cys Lys Trp
 35 40 45

Asp Leu Leu Thr Lys Gln Trp Val Cys His Gly Leu Gly Gly Gly Gly
 50 55 60

Gly Val Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 65 70 75 80

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 85 90 95

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 100 105 110

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 115 120 125

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 130 135 140

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 145 150 155 160

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 165 170 175

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 180 185 190

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 195 200 205

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 210 215 220

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
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<210> 125
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Consensus Sequence

<220>
<221> misc_feature
<222> (1, 2 and)..(3)
<223> X at (Pos 1, 2, 3) are absent or are amino acid residues (with on
      e of X1, X2, and X3 preferred to be C when one of X12,
      X13, and
      d X14 is C);

<220>
<221> misc_feature
<222> (7)..(7)
<223> X at (Pos 7) is an amino acid residue (L preferred);

<220>
<221> misc_feature
<222> (9)..(9)
<223> X at (Pos 9) is T or I (T preferred);

<220>
<221> misc_feature
<222> (12)..(12)
<223> X at (Pos 12) is C, a neutral hydrophobic residue, or a basic res
      idue (W, C, or R
      preferred);

<220>
<221> misc_feature
<222> (13)..(13)
<223> X at (Pos 13) is C, a neutral hydrophobic residue or is absent (V

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preferred);

<220>

<221> misc_feature

<222> (14)..(14)

<223> X at (Pos 14) is any amino acid residue or is absent.

<400> 125

Xaa	Xaa	Xaa	Lys	Trp	Asp	Xaa	Leu	Xaa	Lys	Gln	Xaa	Xaa	Xaa
1			5						10				

<210> 126

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 126

Tyr	Lys	Gly	Arg	Gln	Met	Trp	Asp	Ile	Leu	Thr	Arg	Ser	Trp	Val	Val
1				5					10					15	

Ser Leu

<210> 127

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 127

Gln	Asp	Val	Gly	Leu	Trp	Trp	Asp	Ile	Leu	Thr	Arg	Ala	Trp	Met	Pro
1				5					10					15	

Asn Ile

<210> 128

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 128

Gln	Asn	Ala	Gln	Arg	Val	Trp	Asp	Leu	Leu	Ile	Arg	Thr	Trp	Val	Tyr
1				5					10					15	

Pro Gln

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<210> 129
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 129

Gly	Trp	Asn	Glu	Ala	Trp	Trp	Asp	Glu	Leu	Thr	Lys	Ile	Trp	Val	Leu
1				5					10					15	

Glu Gln

<210> 130
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 130

Arg	Ile	Thr	Cys	Asp	Thr	Trp	Asp	Ser	Leu	Ile	Lys	Lys	Cys	Val	Pro
1			5						10					15	

Gln Ser

<210> 131
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 131

Gly	Ala	Ile	Met	Gln	Phe	Trp	Asp	Ser	Leu	Thr	Lys	Thr	Trp	Leu	Arg
1				5					10					15	

Gln Ser

<210> 132
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 132

Trp	Leu	His	Ser	Gly	Trp	Trp	Asp	Pro	Leu	Thr	Lys	His	Trp	Leu	Gln
1				5					10					15	

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Lys Val

<210> 133
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 133

Ser Glu Trp Phe Phe Trp Phe Asp Pro Leu Thr Arg Ala Gln Leu Lys
1 5 10 15

Phe Arg

<210> 134
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains
<400> 134

Gly Val Trp Phe Trp Trp Phe Asp Pro Leu Thr Lys Gln Trp Thr Gln
1 5 10 15

Ala Gly

<210> 135
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 135

Met Gln Cys Lys Gly Tyr Tyr Asp Ile Leu Thr Lys Trp Cys Val Thr
1 5 10 15

Asn Gly

<210> 136
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains
<400> 136

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Leu Trp Ser Lys Glu Val Trp Asp Ile Leu Thr Lys Ser Trp Val Ser
1 5 10 15

Gln Ala

<210> 137
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 137

Lys Ala Ala Gly Trp Trp Phe Asp Trp Leu Thr Lys Val Trp Val Pro
1 5 10 15

Ala Pro

<210> 138
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 138

Ala Tyr Gln Thr Trp Phe Trp Asp Ser Leu Thr Arg Leu Trp Leu Ser
1 5 10 15

Thr Thr

<210> 139
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
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<400> 139

Ser Gly Gln His Phe Trp Trp Asp Leu Leu Thr Arg Ser Trp Thr Pro
1 5 10 15

Ser Thr

<210> 140
<211> 18
<212> PRT
<213> Artificial Sequence

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<220>

<223> Preferred TALL-1 modulating domains

<400> 140

Leu Gly Val Gly Gln Lys Trp Asp Pro Leu Thr Lys Gln Trp Val Ser
 1 5 10 15

Arg Gly

<210> 141

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 141

Val Gly Lys Met Cys Gln Trp Asp Pro Leu Ile Lys Arg Thr Val Cys
 1 5 10 15

Val Gly

<210> 142

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 142

Cys Arg Gln Gly Ala Lys Phe Asp Leu Leu Thr Lys Gln Cys Leu Leu
 1 5 10 15

Gly Arg

<210> 143

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 143

Gly Gln Ala Ile Arg His Trp Asp Val Leu Thr Lys Gln Trp Val Asp
 1 5 10 15

Ser Gln

<210> 144

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<211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 144

Arg Gly Pro Cys Gly Ser Trp Asp Leu Leu Thr Lys His Cys Leu Asp
 1 5 10 15

Ser Gln

<210> 145
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 145

Trp Gln Trp Lys Gln Gln Trp Asp Leu Leu Thr Lys Gln Met Val Trp
 1 5 10 15

Val Gly

<210> 146
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 146

Pro Ile Thr Ile Cys Arg Lys Asp Leu Leu Thr Lys Gln Val Val Cys
 1 5 10 15

Leu Asp

<210> 147
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 147

Lys Thr Cys Asn Gly Lys Trp Asp Leu Leu Thr Lys Gln Cys Leu Gln
 1 5 10 15

Gln Ala

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<210> 148
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 148

Lys Cys Leu Lys Gly Lys Trp Asp Leu Leu Thr Lys Gln Cys Val Thr
1 5 10 15

Glu Val

<210> 149
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 149

Arg Cys Trp Asn Gly Lys Trp Asp Leu Leu Thr Lys Gln Cys Ile His
1 5 10 15

Pro Trp

<210> 150
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 150

Asn Arg Asp Met Arg Lys Trp Asp Pro Leu Ile Lys Gln Trp Ile Val
1 5 10 15

Arg Pro

<210> 151
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 151

Gln Ala Ala Ala Ala Thr Trp Asp Leu Leu Thr Lys Gln Trp Leu Val

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1 5 10 15

Pro Pro

<210> 152
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 152

Pro Glu Gly Gly Pro Lys Trp Asp Pro Leu Thr Lys Gln Phe Leu Pro
 1 5 10 15

Pro Val

<210> 153
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 153

Gln Thr Pro Gln Lys Lys Trp Asp Leu Leu Thr Lys Gln Trp Phe Thr
 1 5 10 15

Arg Asn

<210> 154
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 154

Ile Gly Ser Pro Cys Lys Trp Asp Leu Leu Thr Lys Gln Met Ile Cys
 1 5 10 15

Gln Thr

<210> 155
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

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<400> 155

Cys Thr Ala Ala Gly Lys Trp Asp Leu Leu Thr Lys Gln Cys Ile Gln
1 5 10 15

Glu Lys

<210> 156

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 156

Val Ser Gln Cys Met Lys Trp Asp Leu Leu Thr Lys Gln Cys Leu Gln
1 5 10 15

Gly Trp

<210> 157

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 157

Val Trp Gly Thr Trp Lys Trp Asp Leu Leu Thr Lys Gln Tyr Leu Pro
1 5 10 15

Pro Gln

<210> 158

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 158

Gly Trp Trp Glu Met Lys Trp Asp Leu Leu Thr Lys Gln Trp Tyr Arg
1 5 10 15

Pro Gln

<210> 159

<211> 18

<212> PRT

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<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 159

Thr	Ala	Gln	Val	Ser	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Leu	Pro
1				5				10						15	

Leu Ala

<210> 160

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 160

Gln	Leu	Trp	Gly	Thr	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Tyr	Ile	Gln
1				5				10						15	

Ile Met

<210> 161

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 161

Trp	Ala	Thr	Ser	Gln	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Val	Gln
1				5				10						15	

Asn Met

<210> 162

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 162

Gln	Arg	Gln	Cys	Ala	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Cys	Val	Leu
1				5				10						15	

Phe Tyr

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<210> 163
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 163

Lys Thr Thr Asp Cys Lys Trp Asp Leu Leu Thr Lys Gln Arg Ile Cys
 1 5 10 15

Gln Val

<210> 164
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 164

Leu Leu Cys Gln Gly Lys Trp Asp Leu Leu Thr Lys Gln Cys Leu Lys
 1 5 10 15

Leu Arg

<210> 165
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 165

Leu Met Trp Phe Trp Lys Trp Asp Leu Leu Thr Lys Gln Leu Val Pro
 1 5 10 15

Thr Phe

<210> 166
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 166

Gln Thr Trp Ala Trp Lys Trp Asp Leu Leu Thr Lys Gln Trp Ile Gly
 1 5 10 15

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Pro Met

<210> 167
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 167

Asn	Lys	Glu	Leu	Leu	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Cys	Arg	Gly
1				5					10					15	

Arg Ser

<210> 168
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 168

Gly	Gln	Lys	Asp	Leu	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Tyr	Val	Arg
1				5					10					15	

Gln Ser

<210> 169
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 169

Pro	Lys	Pro	Cys	Gln	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Cys	Leu	Gly
1				5					10					15	

Ser Val

<210> 170
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains
 <400> 170

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Gly Gln Ile Gly Trp Lys Trp Asp Leu Leu Thr Lys Gln Trp Ile Gln
 1 5 10 15

Thr Arg

<210> 171
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 171

Val Trp Leu Asp Trp Lys Trp Asp Leu Leu Thr Lys Gln Trp Ile His
 1 5 10 15

Pro Gln

<210> 172
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 172

Gln Glu Trp Glu Tyr Lys Trp Asp Leu Leu Thr Lys Gln Trp Gly Trp
 1 5 10 15

Leu Arg

<210> 173
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 173

His Trp Asp Ser Trp Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Val
 1 5 10 15

Gln Ala

<210> 174
 <211> 18
 <212> PRT
 <213> Artificial Sequence

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<220>

<223> Preferred TALL-1 modulating domains

<400> 174

Thr	Arg	Pro	Leu	Gln	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Leu	Arg
1				5					10					15	

Val Gly

<210> 175

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 175

Ser	Asp	Gln	Trp	Gln	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Phe	Trp
1				5					10					15	

Asp Val

<210> 176

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 176

Gln	Gln	Thr	Phe	Met	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Trp	Ile	Arg
1				5					10					15	

Arg His

<210> 177

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Preferred TALL-1 modulating domains

<400> 177

Gln	Gly	Glu	Cys	Arg	Lys	Trp	Asp	Leu	Leu	Thr	Lys	Gln	Cys	Phe	Pro
1				5					10					15	

Gly Gln

<210> 178

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<211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 178

Gly Gln Met Gly Trp Arg Trp Asp Pro Leu Ile Lys Met Cys Leu Gly
 1 5 10 15

Pro Ser

<210> 179
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 179

Gln Leu Asp Gly Cys Lys Trp Asp Leu Leu Thr Lys Gln Lys Val Cys
 1 5 10 15

Ile Pro

<210> 180
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 180

His Gly Tyr Trp Gln Lys Trp Asp Leu Leu Thr Lys Gln Trp Val Ser
 1 5 10 15

Ser Glu

<210> 181
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 181

His Gln Gly Gln Cys Gly Trp Asp Leu Leu Thr Arg Ile Tyr Leu Pro
 1 5 10 15

Cys His

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<210> 182
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 182

Leu His Lys Ala Cys Lys Trp Asp Leu Leu Thr Lys Gln Cys Trp Pro
1 5 10 15

Met Gln

<210> 183
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 183

Gly Pro Pro Gly Ser Val Trp Asp Leu Leu Thr Lys Ile Trp Ile Gln
1 5 10 15

Thr Gly

<210> 184
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 184

Ile Thr Gln Asp Trp Arg Phe Asp Thr Leu Thr Arg Leu Trp Leu Pro
1 5 10 15

Leu Arg

<210> 185
<211> 18
<212> PRT
<213> Artificial Sequence

<220>
<223> Preferred TALL-1 modulating domains

<400> 185

Gln Gly Gly Phe Ala Ala Trp Asp Val Leu Thr Lys Met Trp Ile Thr
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1 5 10 15

Val Pro

<210> 186
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 186

Gly His Gly Thr Pro Trp Trp Asp Ala Leu Thr Arg Ile Trp Ile Leu
 1 5 10 15

Gly Val

<210> 187
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 187

Val Trp Pro Trp Gln Lys Trp Asp Leu Leu Thr Lys Gln Phe Val Phe
 1 5 10 15

Gln Asp

<210> 188
 <211> 19
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Preferred TALL-1 modulating domains

<400> 188

Trp Gln Gln Trp Ser Trp Lys Trp Asp Leu Leu Thr Arg Gln Tyr Ile
 1 5 10 15

Ser Ser Ser

<210> 189
 <211> 882
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TALL-1 12-3 tandem dimer

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<400> 189
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catatgctgc cgggttgtaa atgggacctg ctgatcaaac agtggggttg tgacccgctg      180
gggtggaggcg gtggggtcga caaaactcac acatgtccac cttgtccage tccggaactc      240
ctgggggggac cgtcagtctt cctcttcccc ccaaaaccca aggacaccct catgatctcc      300
cggacccttg aggtcacatg cgtgggtggtg gacgtgagcc acgaagaccc tgaggtcaag      360
ttcaactggt acgtggacgg cgtggagggtg cataatgcc aagacaaagcc gcgggaggag      420
cagtacaaca gcacgtaccg tgtggtcagc gtcctcaccg tcctgcacca ggactggctg      480
aatggcaagg agtacaagtg caaggtctcc aacaaagccc tcccagcccc catcgagaaa      540
accatctcca aagccaaagg gcagccccga gaaccacagg tgtacaccct gccccatcc      600
cgggatgagc tgaccaagaa ccaggtcagc ctgacctgcc tggtaaaagg cttctatccc      660
agcgacatcg ccgtggagtg ggagagcaat gggcagccgg agaacaacta caagaccacg      720
cctcccgtgc tggactccga cggctccttc ttctctaca gcaagctcac cgtggacaag      780
agcagggtggc agcaggggaa cgtcttctca tgctccgtga tgcattgaggc tctgcacaac      840
cactacacgc agaagagcct ctccctgtct ccgggtaaat aa                        882

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<210> 190
<211> 23
<212> PRT
<213> Artificial Sequence

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<220>
<223> Preferred linker

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<400> 190

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Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly
1           5           10          15

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Ser Gly Ser Ala Thr Gly Met
20

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<210> 191
<211> 23
<212> PRT
<213> Artificial Sequence

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<220>
<223> Preferred linker

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<400> 191

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Gly Ser Gly Ser Ala Thr Gly Gly Ser Gly Ser Thr Ala Ser Ser Gly
1           5           10          15

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Ser Gly Ser Ala Thr Gly Ser
20

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Ser Gly Ser Ala Thr Xaa Xaa Gly Ser Gly Ser Ala Thr Gly Gly Ser
 20 25 30

Gly Ser Thr Ala Ser Ser Gly Ser Gly Ser Ala Thr Xaa Xaa
 35 40 45

<210> 195
 <211> 38
 <212> PRT
 <213> Human

<400> 195

Met Arg Arg Gly Pro Arg Ser Leu Arg Gly Arg Asp Ala Pro Val Pro
 1 5 10 15

Thr Pro Cys Val Pro Thr Glu Cys Tyr Asp Leu Leu Val Arg Lys Cys
 20 25 30

Val Asp Cys Arg Leu Leu
 35

<210> 196
 <211> 41
 <212> PRT
 <213> Human

<400> 196

Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala Phe Cys Arg
 1 5 10 15

Ser Leu Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp His Leu Leu
 20 25 30

Arg Asp Cys Ile Ser Cys Ala Ser Ile
 35 40

<210> 197
 <211> 42
 <212> PRT
 <213> Human

<400> 197

Phe Val Ser Pro Ser Gln Glu Ile Arg Gly Arg Phe Arg Arg Met Leu
 1 5 10 15

Gln Met Ala Gly Gln Cys Ser Gln Asn Glu Tyr Phe Asp Ser Leu Leu
 20 25 30

His Ala Cys Ile Pro Cys Gln Leu Arg Cys
 35 40

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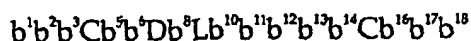
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- (74) Agents: ODRE, Steven et al.; Amgen, Inc., One Amgen Center Drive, M/S 27-4-A, Thousand Oaks, CA 91320-1799 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

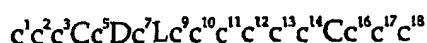
(54) Title: PEPTIDES AND RELATED MOLECULES THAT BIND TO TALL-1



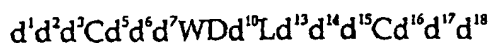
(SEQ. ID. NO: 100),



(SEQ. ID. NO: 104)



(SEQ. ID. NO: 105)



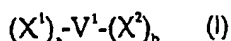
(SEQ. ID. NO: 106)



(SEQ. ID. NO: 107)



(SEQ. ID. NO: 109)



(57) Abstract: The present invention concerns therapeutic agents that modulate the activity of TALL-1. In accordance with the present invention, modulators of TALL-1 may comprise an amino acid sequence Dz^2Lz^4 wherein z^2 is an amino acid residue and z^4 is threonyl or isoleucyl. Exemplary molecules comprise a sequence of the formulae $a^1a^2a^3CDa^6La^8a^9a^{10}Ca^{12}a^{13}a^{14}$ (SEQ.ID.NO:100), $b^1b^2b^3Cb^5b^6Db^8Lb^{10}b^{11}b^{12}b^{13}b^{14}Cb^{16}b^{17}b^{18}$ (SEQ.ID.NO:104), $c^1c^2c^3Cc^5Dc^7Lc^9c^{10}c^{11}c^{12}c^{13}c^{14}Cc^{16}c^{17}c^{18}$ (SEQ.ID.NO:105), $d^1d^2d^3Cd^5d^6d^7WDd^{10}Ld^{13}d^{14}d^{15}Cd^{16}d^{17}d^{18}$ (SEQ.ID.NO:106), $e^1e^2e^3Ce^5e^6e^7De^9Le^{11}Ke^{13}Ce^{15}e^{16}e^{17}e^{18}$ (SEQ.ID.NO:107), $f^1f^2f^3Kf^5Df^7Lf^9Qf^{12}f^{13}f^{14}$ (SEQ.ID. NO:109) wherein the substituents are as defined in the specification. The invention further comprises compositions of matter of the formula $(X^1)_n-V^1-(X^2)_m$ wherein V^1 is a vehicle that is covalently attached to one or more of the above TALL-1 modulating compositions of matter. The vehicle and the TALL-1 modulating composition of matter may be linked through the N- or C-terminus of the TALL-1 modulating portion. The preferred vehicle is an Fc domain, and the preferred Fc domain is an IgG Fc domain.



Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/15273

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/52, 14/525; A61K 38/19; C12N 5/10, 15/28

US CL : 530/351, 402; 514/2, 8, 12; 536/23.5; 435/69.1, 71.1, 471, 320.1, 325, 252.3, 254.11

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/351, 402; 514/2, 8, 12; 536/23.5; 435/69.1, 71.1, 471, 320.1, 325, 252.3, 254.11

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONEElectronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database PNAS, SHU, H.-B. et al. B cell maturation protein is a receptor for the tumor necrosis factor family member TALL-1. Proc. Natl. Acad. Sci. USA. 01 August 2000, Vol. 97, No. 16, pages 9156-9161.	1-62
A	Database PNAS, KHARE et al. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc. Natl. Acad. Sci. USA. 28 March 2000, Vol. 97, No. 7, pages 3370-3375.	1-62

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:		"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier application or patent published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

17 March 2003 (17.03.2003)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

PCT/US02/15273

Continuation of B. FIELDS SEARCHED Item 3:

CAS ONLINE, MEDLINE, CAPLUS, EMBASE, USPATFULL

search terms: TALL-1, binding composition, ligand, hybrid, chimera, DNA, expression vector, host cell, administering, treatment, therapy

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International Bureau



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- (51) International Patent Classification⁷: C07K (74) Agents: ODRE, Steven et al.; Amgen, Inc., One Amgen Center Drive, M/S 27-4-A, Thousand Oaks, CA 91320-1799 (US).
- (21) International Application Number: PCT/US02/15273
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- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 60/290,196 11 May 2001 (11.05.2001) US
- (71) Applicant: AMGEN, INC. [US/US]; One Amgen Center Drive, Thousand Oaks, CA 91320-1799 (US).
- (72) Inventors: MIN, Hosung; 3875 Conner Court, Newbury Park, CA 91320 (US). HSU, Hailing; 11623 Blossomwood, Moorpark, CA 93021 (US).
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: PEPTIDES AND RELATED MOLECULES THAT BIND TO TALL-1

$a^1a^2a^3CDa^6La^8a^9a^{10}Ca^{12}a^{13}a^{14}$

(SEQ. ID. NO: 100),

$b^1b^2b^3Cb^5b^6Db^8Lb^{10}b^{11}b^{12}b^{13}b^{14}Cb^{16}b^{17}b^{18}$

(SEQ. ID. NO: 104)

$c^1c^2c^3Cc^5Dc^7Lc^9c^{10}c^{11}c^{12}c^{13}c^{14}Cc^{16}c^{17}c^{18}$

(SEQ. ID. NO: 105)

$d^1d^2d^3Cd^5d^6d^7WDd^{10}Ld^{13}d^{14}d^{15}Cd^{16}d^{17}d^{18}$

(SEQ. ID. NO: 106)

$e^1e^2e^3Ce^5e^6e^7De^9Le^{11}Ke^{13}Ce^{15}e^{16}e^{17}e^{18}$

(SEQ. ID. NO: 107)

$f^1f^2f^3Kf^5Df^7Lf^9f^{10}Qf^{12}f^{13}f^{14}$

(SEQ. ID NO: 109)

(57) Abstract: The present invention concerns therapeutic agents that modulate the activity of TALL-1. In accordance with the present invention, modulators of TALL-1 may comprise an amino acid sequence Dz^2Lz^4 wherein z^2 is an amino acid residue and z^4 is threonyl or isoleucyl. Exemplary molecules comprise a sequence of the formulae $a^1a^2a^3CDa^6La^8a^9a^{10}Ca^{12}a^{13}a^{14}$ (SEQ.ID.NO:100), $b^1b^2b^3Cb^5b^6Db^8Lb^{10}b^{11}b^{12}b^{13}b^{14}Cb^{16}b^{17}b^{18}$ (SEQ.ID.NO:104), $c^1c^2c^3Cc^5Dc^7Lc^9c^{10}c^{11}c^{12}c^{13}c^{14}Cc^{16}c^{17}c^{18}$ (SEQ.ID.NO:105), $d^1d^2d^3Cd^5d^6d^7WDd^{10}Ld^{13}d^{14}d^{15}Cd^{16}d^{17}d^{18}$ (SEQ.ID.NO:106), $e^1e^2e^3Ce^5e^6e^7De^9Le^{11}Ke^{13}Ce^{15}e^{16}e^{17}e^{18}$ (SEQ.ID.NO:107), $f^1f^2f^3Kf^5Df^7Lf^9f^{10}Qf^{12}f^{13}f^{14}$ (SEQ.ID NO:109) wherein the substituents are as defined in the specification. The invention further comprises compositions of matter of the formula $(X^1)_a-V^1-(X^2)_b$ wherein V^1 is a vehicle that is covalently attached to one or more of the above TALL-1 modulating compositions of matter. The vehicle and the TALL-1 modulating composition of matter may be linked through the N- or C-terminus of the TALL-1 modulating portion. The preferred vehicle is an Fc domain, and the preferred Fc domain is an IgG Fc domain.

$(X^1)_a-V^1-(X^2)_b$ (I)

WO 02/092620 A2



Published:

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

PEPTIDES AND RELATED MOLECULES THAT BIND TO TALL-1

This application is related to U.S. provisional application no. 60/290,196,
5 filed May 11, 2001, which is hereby incorporated by reference.

Background of the Invention

After years of study in necrosis of tumors, tumor necrosis factors (TNFs) α and β were finally cloned in 1984. The ensuing years witnessed
10 the emergence of a superfamily of TNF cytokines, including fas ligand (FasL), CD27 ligand (CD27L), CD30 ligand (CD30L), CD40 ligand (CD40L), TNF-related apoptosis-inducing ligand (TRAIL, also designated AGP-1), osteoprotegerin binding protein (OPG-BP or OPG ligand), 4-1BB ligand, LIGHT, APRIL, and TALL-1. Smith *et al.* (1994), *Cell* 76: 959-962;
15 Lacey *et al.* (1998), *Cell* 93: 165-176; Chichepotiche *et al.* (1997), *J. Biol. Chem.* 272: 32401-32410; Mauri *et al.* (1998), *Immunity* 8: 21-30; Hahne *et al.* (1998), *J. Exp. Med.* 188: 1185-90; Shu *et al.* (1999), *J. Leukocyte Biology* 65: 680-3. This family is unified by its structure, particularly at the C-terminus. In addition, most members known to date are expressed in
20 immune compartments, although some members are also expressed in other tissues or organs, as well. Smith *et al.* (1994), *Cell* 76: 959-62. All ligand members, with the exception of LT- α , are type II transmembrane proteins, characterized by a conserved 150 amino acid region within C-terminal extracellular domain. Though restricted to only 20-25% identity,
25 the conserved 150 amino acid domain folds into a characteristic β -pleated sheet sandwich and trimerizes. This conserved region can be proteolytically released, thus generating a soluble functional form. Banner *et al.* (1993), *Cell* 73: 431-445.

Many members within this ligand family are expressed in lymphoid enriched tissues and play important roles in the immune system development and modulation. Smith *et al.* (1994). For example, TNF α is mainly synthesized by macrophages and is an important mediator for inflammatory responses and immune defenses. Tracey & Cerami (1994), *Ann. Rev. Med.* 45: 491-503. Fas-L, predominantly expressed in activated T cell, modulates TCR-mediated apoptosis of thymocytes. Nagata, S. & Suda, T. (1995) *Immunology Today* 16: 39-43; Castrim *et al.* (1996), *Immunity* 5: 617-27. CD40L, also expressed by activated T cells, provides an essential signal for B cell survival, proliferation and immunoglobulin isotype switching. Noelle (1996), *Immunity* 4: 415-9.

The cognate receptors for most of the TNF ligand family members have been identified. These receptors share characteristic multiple cysteine-rich repeats within their extracellular domains, and do not possess catalytic motifs within cytoplasmic regions. Smith *et al.* (1994). The receptors signal through direct interactions with death domain proteins (e.g. TRADD, FADD, and RIP) or with the TRAF proteins (e.g. TRAF2, TRAF3, TRAF5, and TRAF6), triggering divergent and overlapping signaling pathways, e.g. apoptosis, NF- κ B activation, or JNK activation. Wallach *et al.* (1999), *Annual Review of Immunology* 17: 331-67. These signaling events lead to cell death, proliferation, activation or differentiation. The expression profile of each receptor member varies. For example, TNFR1 is expressed on a broad spectrum of tissues and cells, whereas the cell surface receptor of OPGL is mainly restricted to the osteoclasts. Hsu *et al.* (1999) *Proc. Natl. Acad. Sci. USA* 96: 3540-5.

A number of research groups have recently identified TNF family ligands with the same or substantially similar sequence. The ligand has been variously named neutrokin α (WO 98/18921, published May 7, 1998), 63954 (WO 98/27114, published June 25, 1998), TL5 (EP 869 180, published October 7, 1998), NTN-2 (WO 98/55620 and WO 98/55621,

published December 10, 1998), TNRL1-alpha (WO 9911791, published March 11, 1999), kay ligand (WO99/12964, published March 18, 1999), and AGP-3 (U.S. Prov. App. Nos. 60/119,906, filed February 12, 1999 and 60/166,271, filed November 18, 1999, respectively); and TALL-1 (WO 5 00/68378, published Nov. 16, 2000). Each of these references is hereby incorporated by reference. Hereinafter, the ligands reported therein are collectively referred to as TALL-1.

TALL-1 is a member of the TNF ligand superfamily that is functionally involved in B cell survival and proliferation. Transgenic mice
10 overexpressing TALL-1 had severe B cell hyperplasia and lupus-like autoimmune disease. Khare *et al.* (2000) *PNAS* 97(7):3370-3375). Both TACI and BCMA serve as cell surface receptors for TALL-1. Gross *et al.* (2000), *Nature* 404: 995-999; Ware (2000), *J. Exp. Med.* 192(11): F35-F37; Ware (2000), *Nature* 404: 949-950; Xia *et al.* (2000), *J. Exp. Med.* 192(1):137-
15 143; Yu *et al.* (2000), *Nature Immunology* 1(3):252-256; Marsters *et al.* (2000), *Current Biology* 10:785-788; Hatzoglou *et al.* (2000) *J. of Immunology* 165:1322-1330; Shu *et al.* (2000) *PNAS* 97(16):9156-9161; Thompson *et al.* (2000) *J. Exp. Med.* 192(1):129-135; Mukhopadhyay *et al.* (1999) *J. Biol. Chem.* 274(23): 15978-81; Shu *et al.* (1999) *J. Leukocyte Biol.*
20 65:680-683; Gruss *et al.* (1995) *Blood* 85(12): 3378-3404; Smith *et al.* (1994), *Cell* 76: 959-962; U.S. Pat. No. 5,969,102, issued October 19, 1999; WO 00/67034, published November 9, 2000; WO 00/40716, published July 13, 2000; WO 99/35170, published July 15, 1999. Both receptors are expressed on B cells and signal through interaction with TRAF proteins. In addition,
25 both TACI and BCMA also bind to another TNF ligand family member, APRIL. Yu *et al.* (2000), *Nature Immunology* 1(3) :252-256. APRIL has also been demonstrated to induce B cell proliferation.

To date, no recombinant or modified proteins employing peptide modulators of TALL-1 have been disclosed. Recombinant and modified

proteins are an emerging class of therapeutic agents. Useful modifications of protein therapeutic agents include combination with the "Fc" domain of an antibody and linkage to polymers such as polyethylene glycol (PEG) and dextran. Such modifications are discussed in detail in a patent
5 application entitled, "Modified Peptides as Therapeutic Agents,"
published WO 00/24782, which is hereby incorporated by reference in its entirety.

A much different approach to development of therapeutic agents is peptide library screening. The interaction of a protein ligand with its
10 receptor often takes place at a relatively large interface. However, as demonstrated for human growth hormone and its receptor, only a few key residues at the interface contribute to most of the binding energy. Clackson *et al.* (1995), *Science* 267: 383-6. The bulk of the protein ligand merely displays the binding epitopes in the right topology or serves
15 functions unrelated to binding. Thus, molecules of only "peptide" length (2 to 40 amino acids) can bind to the receptor protein of a given large protein ligand. Such peptides may mimic the bioactivity of the large protein ligand ("peptide agonists") or, through competitive binding, inhibit the bioactivity of the large protein ligand ("peptide antagonists").

20 Phage display peptide libraries have emerged as a powerful method in identifying such peptide agonists and antagonists. See, for example, Scott *et al.* (1990), *Science* 249: 386; Devlin *et al.* (1990), *Science* 249: 404; U.S. Pat. No. 5,223,409, issued June 29, 1993; U.S. Pat. No. 5,733,731, issued March 31, 1998; U.S. Pat. No. 5,498,530, issued March 12,
25 1996; U.S. Pat. No. 5,432,018, issued July 11, 1995; U.S. Pat. No. 5,338,665, issued August 16, 1994; U.S. Pat. No. 5,922,545, issued July 13, 1999; WO 96/40987, published December 19, 1996; and WO 98/15833, published April 16, 1998 (each of which is incorporated by reference in its entirety). In such libraries, random peptide sequences are displayed by fusion with

coat proteins of filamentous phage. Typically, the displayed peptides are affinity-eluted against an immobilized target protein. The retained phages may be enriched by successive rounds of affinity purification and repropagation. The best binding peptides may be sequenced to identify
5 key residues within one or more structurally related families of peptides. See, e.g., Cwirla *et al.* (1997), *Science* 276: 1696-9, in which two distinct families were identified. The peptide sequences may also suggest which residues may be safely replaced by alanine scanning or by mutagenesis at the DNA level. Mutagenesis libraries may be created and screened to
10 further optimize the sequence of the best binders. Lowman (1997), *Ann. Rev. Biophys. Biomol. Struct.* 26: 401-24.

Structural analysis of protein-protein interaction may also be used to suggest peptides that mimic the binding activity of large protein ligands. In such an analysis, the crystal structure may suggest the identity
15 and relative orientation of critical residues of the large protein ligand, from which a peptide may be designed. See, e.g., Takasaki *et al.* (1997), *Nature Biotech.* 15: 1266-70. These analytical methods may also be used to investigate the interaction between a receptor protein and peptides selected by phage display, which may suggest further modification of the
20 peptides to increase binding affinity.

Other methods compete with phage display in peptide research. A peptide library can be fused to the carboxyl terminus of the *lac* repressor and expressed in *E. coli*. Another *E. coli*-based method allows display on the cell's outer membrane by fusion with a peptidoglycan-associated
25 lipoprotein (PAL). Hereinafter, these and related methods are collectively referred to as "*E. coli* display." In another method, translation of random RNA is halted prior to ribosome release, resulting in a library of polypeptides with their associated RNA still attached. Hereinafter, this and related methods are collectively referred to as "ribosome display."

Other methods employ peptides linked to RNA; for example, PROfusion technology, Phylos, Inc. See, for example, Roberts & Szostak (1997), Proc. Natl. Acad. Sci. USA, 94: 12297-303. Hereinafter, this and related methods are collectively referred to as "RNA-peptide screening." Chemically

5 derived peptide libraries have been developed in which peptides are immobilized on stable, non-biological materials, such as polyethylene rods or solvent-permeable resins. Another chemically derived peptide library uses photolithography to scan peptides immobilized on glass slides. Hereinafter, these and related methods are collectively referred to as

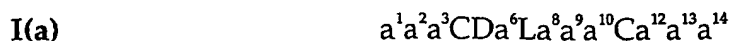
10 "chemical-peptide screening." Chemical-peptide screening may be advantageous in that it allows use of D-amino acids and other unnatural analogues, as well as non-peptide elements. Both biological and chemical methods are reviewed in Wells & Lowman (1992), Curr. Opin. Biotechnol. 3: 355-62. Conceptually, one may discover peptide mimetics of any

15 protein using phage display, RNA-peptide screening, and the other methods mentioned above.

Summary of the Invention

The present invention concerns therapeutic agents that modulate the activity of TALL-1. In accordance with the present invention,

20 modulators of TALL-1 may comprise an amino acid sequence Dz^2Lz^4 (SEQ ID NO: 108) wherein z^2 is an amino acid residue and z^4 is threonyl or isoleucyl. Such modulators of TALL-1 comprise molecules of the following formulae:



25 (SEQ. ID. NO: 100)

wherein:

- a^1, a^2, a^3 are each independently absent or amino acid residues;
- a^6 is an amino acid residue;
- a^9 is a basic or hydrophobic residue;
- 30 a^8 is threonyl or isoleucyl;

(19) World Intellectual Property Organization
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28 November 2002 (28.11.2002)

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60/293,343 24 May 2001 (24.05.2001) US
- (71) Applicant: **ZYMOGENETICS, INC.** [US/US]; 1201 Eastlake Avenue East, Seattle, WA 98102 (US).
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- (72) Inventors: **RIXON, Mark, W.**; 3724 248th Place SE, Issaquah, WA 98029 (US). **GROSS, Jane, A.**; 4258 NE 74th Street, Seattle, WA 98115 (US).
- (74) Agent: **JONES, Phillip, B., C.**; ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA 98102 (US).

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WO 02/094852 A2

(54) Title: TACI-IMMUNOGLOBULIN FUSION PROTEINS

(57) Abstract: Molecules that interfere with the binding of a tumor necrosis factor receptor with its ligand, such as a soluble receptor, have proven usefulness in both basic research and as therapeutics. The present invention provides improved soluble transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI) receptors.

TACI-IMMUNOGLOBULIN FUSION PROTEINS

5

TECHNICAL FIELD

The present invention relates generally to improved fusion proteins comprising a tumor necrosis factor receptor moiety and an immunoglobulin moiety. In particular, the present invention relates to improved TACI-immunoglobulin fusion proteins.

BACKGROUND OF THE INVENTION

Cytokines are soluble, small proteins that mediate a variety of biological effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al.*, *Annu. Rev. Biochem.* 59:783 (1990); Mosmann, *Curr. Opin. Immunol.* 3:311 (1991); Paul and Seder, *Cell* 76:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al.*, *J. Immunol.* 155:5483 (1995); Fossiez *et al.*, *J. Exp. Med.* 183:2593 (1996)).

Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor chains responsible for binding and/or transducing the effect of interferons are members of the type II cytokine receptor family, based upon a characteristic 200 residue extracellular domain.

Cellular interactions, which occur during an immune response, are regulated by members of several families of cell surface receptors, including the tumor necrosis factor receptor (TNFR) family. The TNFR family consists of a number of integral membrane glycoprotein receptors many of which, in conjunction with their respective ligands, regulate interactions between different hematopoietic cell lineages

(see, for example, Cosman, *Stem Cells* 12:440 (1994); Wajant *et al.*, *Cytokine Growth Factor Rev.* 10:15 (1999); Yeh *et al.*, *Immunol. Rev.* 169:283 (1999); Idriss and Naismith, *Microsc. Res. Tech.* 50:184 (2000)).

One such receptor is TACI, transmembrane activator and CAML-
interactor (von Bülow and Bram, *Science* 228:138 (1997); Bram and von Bülow, U.S.
Patent No. 5,969,102 (1999)). TACI is a membrane bound receptor, which has an
extracellular domain containing two cysteine-rich pseudo-repeats, a transmembrane
domain and a cytoplasmic domain that interacts with CAML (calcium-modulator and
cyclophilin ligand), an integral membrane protein located at intracellular vesicles which
is a co-inducer of NF-AT activation when overexpressed in Jurkat cells. TACI is
associated with B cells and a subset of T cells. Nucleotide sequences that encode TACI
and its corresponding amino acid sequence are provided herein as SEQ ID NOs: 1 and 2,
respectively

The TACI receptor binds two members of the tumor necrosis factor
(TNF) ligand family. One ligand is variously designated as ZTNF4, "BAFF,"
"neutrokin- α ," "BLyS," "TALL-1," and "THANK" (Yu *et al.*, international publication
No. WO98/18921 (1998), Moore *et al.*, *Science* 285:269 (1999); Mukhopadhyay *et al.*, *J.*
Biol. Chem. 274:15978 (1999); Schneider *et al.*, *J. Exp. Med.* 189:1747 (1999); Shu
et al., *J. Leukoc. Biol.* 65:680 (1999)). The amino acid sequence of ZTNF4 is provided as
SEQ ID NO:3. The other ligand has been designated as "ZTNF2," "APRIL" and "TNRF
death ligand-1" (Hahne *et al.*, *J. Exp. Med.* 188:1185 (1998); Kelly *et al.*, *Cancer Res.*
60:1021 (2000)). The amino acid sequence of ZTNF2 is provided as SEQ ID NO:4.
Both ligands are also bound by the B-cell maturation receptor (BCMA) (Gross *et al.*,
Nature 404:995 (2000)). The nucleotide and amino acid sequence of BCMA are
provided as SEQ ID NO:26 and SEQ ID NO:27, respectively.

The demonstrated *in vivo* activities of tumor necrosis factor receptors
illustrate the clinical potential of soluble forms of the receptor. Soluble forms of the
TACI receptor have been generated as immunoglobulin fusion proteins. Initial versions
resulted in low-expressing, heterogeneous protein. The heterogeneity was observed at
the TACI amino terminus, at the Fc carboxyl terminus, and in the TACI stalk region. A
need therefore exists for pharmaceutically useful TACI receptor compositions.

BRIEF SUMMARY OF THE INVENTION

The present invention provides improved TACI-immunoglobulin fusion
proteins suitable as therapeutic compounds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence of human TACI. The locations of the cysteine-rich pseudo-repeats are indicated by shading, the transmembrane domain is boxed, and the stalk region is indicated by hash marks.

5 Figure 2 is a schematic diagram of an immunoglobulin of the IgG1 subclass. C_L: light chain constant region; C_{H1}, C_{H2}, C_{H3}: heavy chain constant regions; V_L: light chain variable region; V_H: heavy chain variable region; CHO: carbohydrate; N: amino terminus; C: carboxyl terminus.

10 Figures 3A, 3B, 3C, and 3D show a comparison of the wild-type human γ 1 constant region Fc amino acid sequence with variants Fc-488, Fc4, Fc5, Fc6, Fc7, and Fc8. The C_{H1} domain of the human γ 1 constant region is not part of the Fc and is therefore not shown. The location of the hinge region, the C_{H2}, and the C_{H3} domains are indicated. The Cys residues normally involved in disulfide bonding to the light chain constant region (LC) and heavy chain constant region (HC) are indicated. A "." symbol indicates identity to wild-type at that position, while "****" indicates the location of the carboxyl terminus, and illustrates the difference in the carboxyl terminus of Fc6 relative to the other Fc versions. Amino acid locations are indicated by EU index positions.

15 Figure 4 shows the specific binding of ¹²⁵I-ZTNF4 with various TACI-Fc constructs. The TACI-Fc fusion proteins had TACI moieties that lacked the first 29 amino acid residues of the amino acid sequence of SEQ ID NO:2. One of the fusion proteins had a TACI moiety with an intact stalk region (TACI (d1-29)-Fc5), whereas three of the TACI-Fc fusion proteins had TACI moieties with various deletions in the stalk region (TACI (d1-29, d107-154)-Fc5; TACI (d1-29, d111-154)-Fc5; TACI (d1-29, d120-154)-Fc5). Experimental details are described in Example 4.

25

DETAILED DESCRIPTION OF THE INVENTION

1. Overview

30 As described below, the present invention provides transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion proteins, and methods for using TACI-immunoglobulin fusion proteins. For example, the present invention provides methods for inhibiting the proliferation of tumor cells, comprising administering to the tumor cells a composition that comprises a TACI-immunoglobulin fusion protein. Such a composition can be administered to cells cultured *in vitro*. Alternatively, the composition can be a pharmaceutical composition that comprises a pharmaceutically acceptable carrier and a

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TACI-immunoglobulin fusion protein, and the pharmaceutical composition can be administered to a subject, which has a tumor. The subject may be a mammalian subject. Administration of the pharmaceutical composition can inhibit, for example, the proliferation of B lymphocytes in a mammalian subject.

5 The present invention also provides methods for inhibiting ZTNF4 activity in a mammal, comprising administering to the mammal a composition that comprises a TACI-immunoglobulin. The ZTNF4 activity can be associated with various diseases and disorders. For example, a pharmaceutical composition that comprises a TACI-immunoglobulin fusion protein can be used to treat an autoimmune disease, such
10 as systemic lupus erythematosus, myasthenia gravis, multiple sclerosis, insulin dependent diabetes mellitus, Crohn's disease, rheumatoid arthritis, polyarticular-course juvenile rheumatoid arthritis, and psoriatic arthritis. Alternatively, a pharmaceutical composition that comprises a TACI-immunoglobulin can be used to treat a disorder such as asthma, bronchitis, emphysema, and end stage renal failure. A pharmaceutical
15 composition comprising a TACI-immunoglobulin can also be used to treat renal disease, such as glomerulonephritis, vasculitis, nephritis, amyloidosis, and pyelonephritis, or a disorder, such as neoplasm, chronic lymphocytic leukemia, multiple myeloma, non-Hodgkin's lymphoma, post-transplantation lymphoproliferative disease, and light chain gammopathy. In certain cases, the ZTNF4 activity can be associated with T cells. A
20 pharmaceutical composition that comprises a TACI-immunoglobulin can also be used to treat a disease or disorder associated with immunosuppression, graft rejection, graft versus host disease, and inflammation. For example, a pharmaceutical composition that comprises a TACI-immunoglobulin can be used to decrease inflammation, and to treat disorders such as joint pain, swelling, anemia, and septic shock.

25 The present invention also provides methods for reducing circulating blood levels of ZTNF4 in a mammalian subject, comprising administering to the mammalian subject a pharmaceutical composition that comprises a pharmaceutically acceptable carrier and a TACI-immunoglobulin fusion protein, wherein administration of the pharmaceutical composition reduces the circulating level of ZTNF4 in the blood of
30 the mammalian subject. As an illustration, the administration of such a pharmaceutical composition can reduce circulating blood levels of ZTNF4 by at least 10%, by at least 20%, by at least 10 to 60%, by at least 20 to 50%, or by at least 30 to 40%, compared with the blood level of ZTNF4 prior to the administration of the pharmaceutical composition. Those of skill in the art can measure circulating levels of ZTNF4.
35 Illustrative methods are described in Example 4 and Example 5.

As described below, illustrative TACI-immunoglobulin fusion proteins comprise:

- (a) a TACI receptor moiety that consists of a fragment of a polypeptide that has the amino acid sequence of amino acid residues 30 to 154 of SEQ ID NO:2, wherein the TACI receptor moiety comprises at least one of (i) amino acid residues 34 to 66 of SEQ ID NO:2, and (ii) amino acid residues 71 to 104 of SEQ ID NO:2, and wherein the TACI receptor moiety binds at least one of ZTNF2 or ZTNF4, and
- (b) an immunoglobulin moiety comprising a constant region of an immunoglobulin.

Suitable TACI receptor moieties include: polypeptides that comprise amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2; polypeptides that comprise amino acid residues 34 to 104 of SEQ ID NO:2; polypeptides that comprise the amino acid sequence of amino acid residues 30 to 110 of SEQ ID NO:2; and polypeptides that have an amino acid sequence consisting of amino acid residues 30 to 110 of SEQ ID NO:2.

The immunoglobulin moiety of a TACI-immunoglobulin fusion protein can comprise a heavy chain constant region, such as a human heavy chain constant region. An IgG1 heavy chain constant region is one example of a suitable heavy chain constant region. An illustrative IgG1 heavy chain constant region is an IgG1 Fc fragment that comprises C_{H2}, and C_{H3} domains. The IgG1 Fc fragment can be a wild-type IgG1 Fc fragment or a mutated IgG1 Fc fragment, such as the Fc fragment comprising the amino acid sequence of SEQ ID NO:33. One exemplary TACI-immunoglobulin fusion protein is a protein that has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

The TACI-immunoglobulin fusion proteins described herein can be multimers, such as dimers.

The present invention also provides nucleic acid molecules that encode a TACI-immunoglobulin fusion protein. An illustrative nucleotide sequence that encodes a TACI-immunoglobulin fusion protein is provided by SEQ ID NO:53.

The present invention also includes TACI soluble receptors that consist of a fragment of a polypeptide that has the amino acid sequence of amino acid residues 30 to 154 of SEQ ID NO:2, wherein the TACI soluble receptor comprises at least one of (i) amino acid residues 34 to 66 of SEQ ID NO:2, and (ii) amino acid residues 71 to 104 of SEQ ID NO:2, and wherein the TACI soluble receptor binds at least one of ZTNF2 or ZTNF4. Additional TACI soluble receptors are described herein as suitable TACI

receptor moieties for TACI-immunoglobulin fusion proteins. Moreover, TACI soluble receptors can be used in methods described for TACI-immunoglobulin fusion proteins.

These and other aspects of the invention will become evident upon
5 reference to the following detailed description and drawings. In addition, various references are identified below.

2. Definitions

In the description that follows, a number of terms are used extensively.
10 The following definitions are provided to facilitate understanding of the invention.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and
15 fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*, α -enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have alterations in sugar moieties and/or
20 in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety
25 include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term
30 "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid molecule having a complementary nucleotide sequence and reverse orientation as
35 compared to a reference nucleotide sequence. For example, the sequence 5'

ATGCACGGG 3' (SEQ ID NO:57) is complementary to 5' CCCGTGCAT 3' (SEQ ID NO:58).

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule. Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene,

proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements (CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol.* 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al.*, *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye *et al.*, *J. Biol. Chem.* 269:25728 (1994)), SP1, cAMP response element binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

"Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.*, endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.*, exogenous DNA). For example, a DNA molecule containing a non-host DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule. Conversely, a heterologous DNA molecule can comprise an endogenous gene operably

linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

5 A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

10 A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

A peptide or polypeptide encoded by a non-host DNA molecule is a "heterologous" peptide or polypeptide.

15 An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

20 A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

30 An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

35 A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example

of a recombinant host is a cell that produces a TACI-Fc fusion protein from an expression vector.

"Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

5 A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a TACI-immunoglobulin fusion protein comprises a TACI receptor moiety and an immunoglobulin moiety. As used herein, a "TACI receptor moiety" is a portion of the extracellular domain of the TACI receptor that binds at least one of ZTNF2 or ZTNF4.
10 The phrase an "immunoglobulin moiety" refers to a polypeptide that comprises a constant region of an immunoglobulin. For example, the immunoglobulin moiety can comprise a heavy chain constant region. The term "TACI-Fc" fusion protein refers to a TACI-immunoglobulin fusion protein in which the immunoglobulin moiety comprises immunoglobulin heavy chain constant regions, C_{H2} and C_{H3}.

15 The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. In the context of TACI receptor binding, the phrase "specifically binds" or "specific binding" refers to the ability of the ligand to competitively bind with the receptor. For example, ZTNF4 specifically binds with the TACI receptor, and this can
20 be shown by observing competition for the TACI receptor between detectably labeled ZTNF4 and unlabeled ZTNF4.

Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*, thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF
25 receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that
30 comprise the complete functional receptor.

In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene
35 transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term "secretory signal sequence" denotes a DNA sequence that encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.*, at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by a splice variant of an mRNA transcribed from a gene.

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity less than 10^9 M^{-1} .

An "antibody fragment" is a portion of an antibody such as F(ab')_2 , F(ab)_2 , Fab' , Fab , and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody.

The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom, which is conjugated to an antibody moiety to produce a conjugate, which is useful for therapy. Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom, which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents, paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-

histidine tract, protein A (Nilsson *et al.*, *EMBO J.* 4:1075 (1985); Nilsson *et al.*, *Methods Enzymol.* 198:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene* 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7952 (1985)), substance P, FLAG peptide (Hopp *et al.*, *Biotechnology* 6:1204 (1988)),
5 streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al.*, *Protein Expression and Purification* 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

A “naked antibody” is an entire antibody, as opposed to an antibody
10 fragment, which is not conjugated with a therapeutic agent. Naked antibodies include both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term “antibody component” includes both an entire antibody and an antibody fragment.

15 An “immunoconjugate” is a conjugate of an antibody component with a therapeutic agent or a detectable label.

A “target polypeptide” or a “target peptide” is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes
20 presented by a major histocompatibility complex molecule to a target polypeptide or target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An “antigenic peptide” is a peptide, which will bind a major histocompatibility complex molecule to form an MHC-peptide complex, which is
25 recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell, which binds or expresses the antigen. The antigenic peptide can be bound in the context of a
30 class I or class II major histocompatibility complex molecule, on an antigen presenting cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain
35 an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an “anti-sense RNA” and a nucleic acid molecule that encodes the anti-sense RNA is termed an

“anti-sense gene.” Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

Due to the imprecision of standard analytical methods, molecular weights and lengths of polymers are understood to be approximate values. When such a value is expressed as “about” X or “approximately” X, the stated value of X will be understood to be accurate to $\pm 10\%$.

3. ***Production of Nucleic Acid Molecules Encoding TACI-Immunoglobulin Proteins***

Figure 1 provides the predicted amino acid sequence of human TACI (von Bülow and Bram, *Science* 278:138 (1997)). The TACI polypeptide contains the following predicted elements: (a) two cysteine-rich pseudo-repeat structures characteristic of tumor necrosis factor ligand binding domains, (b) a 62 amino acid “stalk region,” which resides between the ligand binding domains and the transmembrane domain, (c) a 20 amino acid transmembrane domain, and (d) a 127 amino acid intracellular domain. The amino acid sequence does not contain a predicted hydrophobic amino terminal signal sequence.

In order to create a soluble form of human TACI for use as an inhibitor of the native ligand:native receptor interaction, a TACI extracellular domain - human immunoglobulin Fc fusion protein was generated. The available human TACI sequence was used as the starting point for designing the fusion protein molecule (von Bülow and Bram, *Science* 278:138 (1997)). This initial construct, designated as “TACI-Fc4,” included amino acid residues 1 through 154 of the TACI polypeptide, and a modified human Fc region, described below. The fusion point of residue 154 was chosen in order to include as much of the stalk region of TACI as possible while not including any potential portion of the predicted transmembrane domain.

Since native TACI polypeptide does not contain an amino terminal signal sequence, an amino terminal signal sequence was added to TACI in order to generate a secreted form of the TACI-Fc fusion protein. The signal sequence was a modified pre-pro sequence from human tissue plasminogen activator. The modifications were included to enhance signal peptidase cleavage and furin protease-specific processing and for that reason this sequence has been referred to as the “optimized tPA (otPA) leader.” The otPA sequence (SEQ ID NO:25) is illustrated below; modified amino acid residues are shaded. The recombinant TACI-Fc fusion protein coding sequence was inserted into an expression vector, which was transfected into Chinese hamster ovary cells.

[illegible]

Transfected Chinese hamster ovary cells produced the TACI-Fc4 protein at a low level of about 0.3 pg/cell/day. Western blot analysis of TACI-Fc protein with goat anti-human IgG Fc antisera revealed two bands, one band was smaller than the expected size of approximately 48 kDa. Amino acid sequence analysis of purified proteins revealed that the smaller band reflected cleavage of TACI fusion proteins at various sites within the TACI stalk region. With reference to SEQ ID NO:2, the major termini were found at amino acid residues 118 and 123, although, proteins were also cleaved at amino acid positions 110, 139, and 141.

25 In addition to heterogeneity caused by cleavage in the stalk region, heterogeneity was also observed at the amino and carboxyl termini. With reference to SEQ ID NO:2, the major amino termini were found at amino acid residues 1, 10, and 13. Differences in the carboxyl terminus reflect the natural heterogeneity of recombinant immunoglobulins and immunoglobulin fusion proteins, which includes the incomplete
30 removal of the carboxyl-terminally-encoded lysine residue. Another source of heterogeneity was found in the variable nature of the carbohydrate structure attached to the Fc encoded immunoglobulin C_{H2} domain.

New versions of TACI-Fc were generated to address the observed heterogeneity. Constructs were designed that included at least one of the following variations in the TACI moiety: (1) portions of the TACI stalk region were deleted, (2) a portion of the TACI stalk region was replaced with a portion of the BCMA stalk region, (3) the arginine residue at position 119 was mutated to eliminate a potential furin cleavage site, (4) the glutamine residue at position 121 was mutated to eliminate a potential furin cleavage site, (5) the arginine residue at position 122 was mutated to eliminate a potential furin cleavage site, (6) amino acid residue at positions 123 and 142 were mutated to amino acid residues found in corresponding positions of murine TACI, (7) the human otPA signal sequence was replaced with a human heavy chain variable

region signal sequence, (8) the valine residue at position 29 was mutated to methionine, and the otPA signal sequence was joined in an amino terminal position to this residue, and (9) the otPA signal sequence was joined in an amino terminal location to the alanine residue at position 30.

Modifications were also introduced in the immunoglobulin moiety. Five classes of immunoglobulin, IgG, IgA, IgM, IgD, and IgE, have been identified in higher vertebrates. IgG, IgD, and IgE proteins are characteristically disulfide linked heterotetramers consisting of two identical heavy chains and two identical light chains. Typically, IgM is found as a pentamer of a tetramer, whereas IgA occurs as a dimer of a tetramer.

IgG comprises the major class as it normally exists as the second most abundant protein found in plasma. In humans, IgG consists of four subclasses, designated IgG1, IgG2, IgG3, and IgG4. As shown in Figure 2, each immunoglobulin heavy chain possesses a constant region that consists of constant region protein domains (C_{H1} , hinge, C_{H2} , and C_{H3}) that are invariant for a given subclass. The heavy chain constant regions of the IgG class are identified with the Greek symbol γ . For example, immunoglobulins of the IgG1 subclass contain a $\gamma 1$ heavy chain constant region.

The Fc fragment, or Fc domain, consists of the disulfide linked heavy chain hinge regions, C_{H2} , and C_{H3} domains. In immunoglobulin fusion proteins, Fc domains of the IgG1 subclass are often used as the immunoglobulin moiety, because IgG1 has the longest serum half-life of any of the serum proteins. Lengthy serum half-life can be a desirable protein characteristic for animal studies and potential human therapeutic use. In addition, the IgG1 subclass possesses the strongest ability to carry out antibody mediated effector functions. The primary effector function that may be most useful in an immunoglobulin fusion protein is the ability for an IgG1 antibody to mediate antibody dependent cellular cytotoxicity. On the other hand, this could be an undesirable function for a fusion protein that functions primarily as an antagonist. Several of the specific amino acid residues that are important for antibody constant region-mediated activity in the IgG1 subclass have been identified. Inclusion or exclusion of these specific amino acids therefore allows for inclusion or exclusion of specific immunoglobulin constant region-mediated activity.

Six versions of a modified human IgG1 Fc were generated for creating Fc fusion proteins. Fc-488 was designed for convenient cloning of a fusion protein containing the human $\gamma 1$ Fc region, and it was constructed using the wild-type human immunoglobulin $\gamma 1$ constant region as a template. Concern about potential deleterious effects due to an unpaired cysteine residue led to the decision to replace the cysteine (amino acid residue 24 of SEQ ID NO:6) that normally disulfide bonds with the

immunoglobulin light chain constant region with a serine residue. An additional change was introduced at the codon encoding EU index position 218 (amino acid residue 22 of SEQ ID NO:6) to introduce a *Bgl*III restriction enzyme recognition site for ease of future DNA manipulations. These changes were introduced into the PCR product encoded on the PCR primers. Due to the location of the *Bgl*III site and in order to complete the Fc hinge region, codons for EU index positions 216 and 217 (amino acid residues 20 and 21 of SEQ ID NO:6) were incorporated in the fusion protein partner sequences.

Fc4, Fc5, and Fc6 contain mutations to reduce effector functions mediated by the Fc by reducing FcγRI binding and complement C1q binding. Fc4 contains the same amino acid substitutions that were introduced into Fc-488. Additional amino acid substitutions were introduced to reduce potential Fc mediated effector functions. Specifically, three amino acid substitutions were introduced to reduce FcγRI binding. These are the substitutions at EU index positions 234, 235, and 237 (amino acid residues 38, 39, and 41 of SEQ ID NO:6). Substitutions at these positions have been shown to reduce binding to FcγRI (Duncan *et al.*, *Nature* 332:563 (1988)). These amino acid substitutions may also reduce FcγRIIa binding, as well as FcγRIII binding (Sondermann *et al.*, *Nature* 406:267 (2000); Wines *et al.*, *J. Immunol.* 164:5313 (2000)).

Several groups have described the relevance of EU index positions 330 and 331 (amino acid residues 134 and 135 of SEQ ID NO:6) in complement C1q binding and subsequent complement fixation (Canfield and Morrison, *J. Exp. Med.* 173:1483 (1991); Tao *et al.*, *J. Exp. Med.* 178:661 (1993)). Amino acid substitutions at these positions were introduced in Fc4 to reduce complement fixation. The C_{H3} domain of Fc4 is identical to that found in the corresponding wild-type polypeptide, except for the stop codon, which was changed from TGA to TAA to eliminate a potential *dam* methylation site when the cloned DNA is grown in *dam* plus strains of *E. coli*.

In Fc5, the arginine residue at EU index position 218 was mutated back to a lysine, because the *Bgl*III cloning scheme was not used in fusion proteins containing this particular Fc. The remainder of the Fc5 sequence matches the above description for Fc4.

Fc6 is identical to Fc5 except that the carboxyl terminal lysine codon has been eliminated. The C-terminal lysine of mature immunoglobulins is often removed from mature immunoglobulins post-translationally prior to secretion from B-cells, or removed during serum circulation. Consequently, the C-terminal lysine residue is typically not found on circulating antibodies. As in Fc4 and Fc5 above, the stop codon in the Fc6 sequence was changed to TAA.

Fc7 is identical to the wild-type γ1 Fc except for an amino acid substitution at EU index position 297 located in the C_{H2} domain. EU index position

Asn-297 (amino acid residue 101 of SEQ ID NO:6) is a site of N-linked carbohydrate attachment. N-linked carbohydrate introduces a potential source of variability in a recombinantly expressed protein due to potential batch-to-batch variations in the carbohydrate structure. In an attempt to eliminate this potential variability, Asn-297 was
5 mutated to a glutamine residue to prevent the attachment of N-linked carbohydrate at that residue position. The carbohydrate at residue 297 is also involved in Fc binding to the FcγRIII (Sondermann *et al.*, *Nature* 406:267 (2000)). Therefore, removal of the carbohydrate should decrease binding of recombinant Fc7 containing fusion proteins to the FcγRs in general. As above, the stop codon in the Fc7 sequence was mutated to
10 TAA.

Fc8 is identical to the wild-type immunoglobulin γ1 region shown in SEQ ID NO:6, except that the cysteine residue at EU index position 220 (amino acid residue 24 of SEQ ID NO:6) was replaced with a serine residue. This mutation eliminated the cysteine residue that normally disulfide bonds with the immunoglobulin light chain
15 constant region.

Illustrative TACI-Fc constructs are described in Table 1.

Table 1
Illustrative TACI-Fc Fusion Protein Constructs

TACI Sequence ^a	Fc Version
TACI ^b	Fc4
TACI ^b	Fc5
TACI ^b	Fcy1
TACI (d107-154)	Fc5
TACI (R119Q)	Fc4
TACI (1-104)-BCMA (42-54) ^c	Fc5
TACI (d143-150)	Fc5
TACI (R142G, d143-150)	Fc5
TACI (R119G, Q121P, R122Q, S123A)	Fc5
TACI(R119G, R122Q)	Fc5
TACI (d1-28, V29M)	Fc6
TACI (d1-29)	Fc6
TACI (d1-29)	Fc5
TACI (d1-29, d107-154)	Fc5
TACI (d1-29, d111-154)	Fc5
TACI (d1-29, d120-154)	Fc5

5 ^a Information about locations, mutations, and deletions of amino acid sequences is provided within parentheses in reference to the amino acid sequence of SEQ ID NO:2.

^b Includes amino acid residues 1 to 154 of SEQ ID NO:2.

^c This construct includes amino acid residues 1 to 104 of SEQ ID NO:2 (TACI) and amino acids 42 to 54 of SEQ ID NO: 27 (BCMA).

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 The TACI-Fc proteins were produced by recombinant Chinese hamster ovary cells, isolated, and analyzed using Western blot analysis and amino acid sequence analysis. Surprisingly, deletion of the first 29 amino acids from the N-terminus of the TACI polypeptide resulted in a ten-fold increase in the production of TACI-Fc fusion proteins by Chinese hamster ovary cells. This deletion also reduced the cleavage of the full-length stalk region. In addition, cleavage within the TACI stalk region was suppressed either by truncating the TACI stalk region, or by replacing the TACI stalk region within another amino acid sequence (e.g., the amino acid sequence of the BCMA stalk region).

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 As described in Example 4, functional analyses of TACI-Fc constructs indicate that fusion proteins TACI (d1-29)-Fc5, TACI (d1-29, d107-154)-Fc5, TACI (d1-29, d111-154)-Fc5, and TACI (d1-29, d120-154)-Fc5 have similar binding affinities for

ZTNF4. However, constructs, TACI (d1-29)-Fc5, TACI (d1-29, d111-154)-Fc5, and TACI (d1-29, d120-154)-Fc5 appear to bind more ZTNF4 per mole of TACI-Fc than construct, TACI (d1-29, d107-154)-Fc5. Depending upon the intended use (*i.e.*, therapeutic, diagnostic, or research), either high capacity or low capacity TACI-Fc fusion proteins can be employed. In addition, a combination of high capacity and low capacity TACI-Fc fusion proteins enables the titration of ZTNF2 or ZTNF4.

The present invention contemplates TACI-immunoglobulin fusion proteins that comprise a TACI receptor moiety consisting of amino acid residues 30 to 106 of SEQ ID NO:2, 30 to 110 of SEQ ID NO:2, 30 to 119 of SEQ ID NO:2, or 30 to 154 of SEQ ID NO:2. The present invention also includes TACI-immunoglobulin fusion proteins that comprise a TACI receptor moiety consisting of amino acid residues 31 to 106 of SEQ ID NO:2, 31 to 110 of SEQ ID NO:2, 31 to 119 of SEQ ID NO:2, or 31 to 154 of SEQ ID NO:2.

More generally, the present invention includes TACI-immunoglobulin fusion proteins, wherein the TACI receptor moiety consists of a fragment of amino acid residues 30 to 154 of SEQ ID NO:2, and wherein the TACI receptor moiety binds at least one of ZTNF2 or ZTNF4. Such fragments comprise a cysteine-rich pseudo-repeat region, and optionally, can include at least one of an N-terminal segment, which resides in an amino-terminal position to the cysteine-rich pseudo-repeat region, and a stalk segment, which resides in a carboxyl-terminal position to the cysteine-rich pseudo-repeat region. Suitable cysteine-rich pseudo-repeat regions include polypeptides that: (a) comprise at least one of amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2, (b) comprise both amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2, or (c) comprise amino acid residues 34 to 104 of SEQ ID NO:2.

Suitable N-terminal segments include the following with reference to SEQ ID NO:2: amino acid residue 33, amino acid residues 32 to 33, amino acid residues 31 to 33, and amino acid residues 30 to 33. Suitable stalk segments include one or more amino acids of amino acid residues 105 to 154 of SEQ ID NO:2. For example, the stalk segment can consist of the following with reference to SEQ ID NO:2: amino acid residue 105, amino acid residues 105 to 106, amino acid residues 105 to 107, amino acid residues 105 to 108, amino acid residues 105 to 109, amino acid residues 105 to 110, amino acid residues 105 to 111, amino acid residues 105 to 112, amino acid residues 105 to 113, amino acid residues 105 to 114, amino acid residues 105 to 115, amino acid residues 105 to 116, amino acid residues 105 to 117, amino acid residues 105 to 118, amino acid residues 105 to 119, amino acid residues 105 to 120, amino acid residues 105 to 121, amino acid residues 105 to 122, amino acid residues 105 to 123, amino acid

residues 105 to 124, amino acid residues 105 to 125, amino acid residues 105 to 126, amino acid residues 105 to 127, amino acid residues 105 to 128, amino acid residues 105 to 129, amino acid residues 105 to 130, amino acid residues 105 to 131, amino acid residues 105 to 132, amino acid residues 105 to 133, amino acid residues 105 to 134, amino acid residues 105 to 135, amino acid residues 105 to 136, amino acid residues 105 to 137, amino acid residues 105 to 138, amino acid residues 105 to 139, amino acid residues 105 to 140, amino acid residues 105 to 141, amino acid residues 105 to 142, amino acid residues 105 to 143, amino acid residues 105 to 144, amino acid residues 105 to 145, amino acid residues 105 to 146, amino acid residues 105 to 147, amino acid residues 105 to 148, amino acid residues 105 to 149, amino acid residues 105 to 150, amino acid residues 105 to 151, amino acid residues 105 to 152, amino acid residues 105 to 153, and amino acid residues 105 to 154.

Additional suitable stalk segments include one or more amino acids of the BCMA stalk region (*i.e.*, amino acid residues 42 to 54 of SEQ ID NO:27. For example, a stalk segment can consist of the following with reference to SEQ ID NO:27: amino acid residue 42, amino acid residues 42 to 43, amino acid residues 42 to 44, amino acid residues 42 to 45, amino acid residues 42 to 46, amino acid residues 42 to 47, amino acid residues 42 to 48, amino acid residues 42 to 49, amino acid residues 42 to 50, amino acid residues 42 to 51, amino acid residues 42 to 52, amino acid residues 42 to 53, and amino acid residues 42 to 54.

More generally, a stalk segment can consist of two to 50 amino acid residues.

The immunoglobulin moiety of a fusion protein described herein comprises at least one constant region of an immunoglobulin. Preferably, the immunoglobulin moiety represents a segment of a human immunoglobulin. The human immunoglobulin sequence can be a wild-type amino acid sequence, or a modified wild-type amino acid sequence, which has at least one of the amino acid mutations discussed above.

The human immunoglobulin amino acid sequence can also vary from wild-type by having one or more mutations characteristic of a known allotypic determinant. Table 2 shows the allotypic determinants of the human IgG γ 1 constant region (Putman, *The Plasma Proteins*, Vol. V, pages 49 to 140 (Academic Press, Inc. 1987)). EU index positions 214, 356, 358, and 431 define the known IgG γ 1 allotypes. Position 214 is in the C_{H1} domain of the IgG γ 1 constant region, and, therefore, does not reside within the Fc sequence. The wild-type Fc sequence of SEQ ID NO:6 includes the G1m(1) and G1m(2-) allotypes. However, the Fc moiety of a TACI-Fc protein can be modified to reflect any combination of these allotypes.

Table 2
Allotypic Determinants of the Human Immunoglobulin γ 1 Constant Region

Allotype	Amino Acid Residue	Amino Acid Position	
		EU Index	SEQ ID NO:6
Glm(1)	Asp, Leu	356, 358	160, 162
Glm(1-)	Glu, Met	356, 358	160, 162
Glm(2)	Gly	431	235
Glm(2-)	Ala	431	235
Glm(3)	Arg	214	---
Glm(3-)	Lys	214	---

5

The examples of TACI-Fc proteins disclosed herein comprise human IgG1 constant regions. However, suitable immunoglobulin moieties also include polypeptides comprising at least one constant region, such as a heavy chain constant region from any of the following immunoglobulins: IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM. Advantageously, immunoglobulin moieties derived from wild-type IgG2 or wild-type IgG4 offer reduced effector function, compared with wild-type IgG1 or wild-type IgG3. The present invention also contemplates fusion proteins that comprise a TACI receptor moiety, as described above, and either albumin or β 2-macroglobulin.

Another type of receptor fusion protein that binds ZTNF2 or ZTNF4 is a BCMA-immunoglobulin fusion protein. Studies have been performed with a BCMA-Fc4 fusion protein in which the BCMA moiety consists of amino acid residues 1 to 48 of SEQ ID NO:27. Surprisingly, pharmacokinetic studies in mice revealed that BCMA-Fc4 fusion protein had a half-life of about 101 hours, whereas a TACI-Fc protein had a half-life of 25 hours. Thus, administration of a BCMA-immunoglobulin fusion protein may be preferred in certain clinical settings. Moreover, a combination of TACI-immunoglobulin and BCMA-immunoglobulin fusion proteins may be advantageous to treat certain conditions. This combination therapy can be achieved by administering TACI-immunoglobulin and BCMA-immunoglobulin fusion proteins, or by administering heterodimers of TACI-immunoglobulin and BCMA-immunoglobulin fusion proteins.

Another type of receptor fusion protein that binds ZTNF4 is an immunoglobulin fusion protein comprising an extracellular domain of a receptor designated as "Ztnfr12." Ztnfr12 amino acid and nucleotide sequences are provided as

SEQ ID NO:59 and SEQ ID NO:60, respectively. Suitable Ztnfr12 receptor moieties include polypeptides comprising amino acid residues 1 to 69 of SEQ ID NO:60, or amino acid residues 19 to 35 of SEQ ID NO:60.

The fusion proteins of the present invention can have the form of single chain polypeptides, dimers, trimers, or multiples of dimers or trimers. Dimers can be homodimers or heterodimers, and trimers can be homotrimers or heterotrimers. Examples of heterodimers include a TACI-immunoglobulin polypeptide with a BCMA-immunoglobulin polypeptide, a TACI-immunoglobulin polypeptide with a Ztnfr12-immunoglobulin polypeptide, and a BCMA-immunoglobulin polypeptide with a Ztnfr12-immunoglobulin polypeptide. Examples of heterotrimers include a TACI-immunoglobulin polypeptide with two BCMA-immunoglobulin polypeptides, a TACI-immunoglobulin polypeptide with two Ztnfr12-immunoglobulin polypeptides, a BCMA-immunoglobulin polypeptide with two Ztnfr12-immunoglobulin polypeptides, two TACI-immunoglobulin polypeptides with a BCMA-immunoglobulin polypeptide, two TACI-immunoglobulin polypeptides with a Ztnfr12-immunoglobulin polypeptide, two BCMA-immunoglobulin polypeptides with a Ztnfr12-immunoglobulin polypeptide, and a trimer of a TACI-immunoglobulin polypeptide, a BCMA-immunoglobulin polypeptide, and a Ztnfr12-immunoglobulin polypeptide.

In such fusion proteins, the TACI receptor moiety can comprise at least one of the following amino acid sequences of SEQ ID NO:2: amino acid residues 30 to 154, amino acid residues 34 to 66, amino acid residues 71 to 104, amino acid residues 47 to 62, and amino acid residues 86 to 100. The BCMA receptor moiety can comprise at least one of the following amino acid sequences of SEQ ID NO:27: amino acid residues 1 to 48, amino acid residues 8 to 41, and amino acid residues 21 to 37. The Ztnfr12 receptor moiety can comprise at least one of the following amino acid sequences of SEQ ID NO:60: amino acid residues 1 to 69, and amino acid residues 19 to 35.

Fusion proteins can be produced using the PCR methods used to construct the illustrative TACI-Fc molecules, which are described in the Examples. However, those of skill in the art can use other standard approaches. For example, nucleic acid molecules encoding TACI, BCMA, Ztnfr12, or immunoglobulin polypeptides can be obtained by screening human cDNA or genomic libraries using polynucleotide probes based upon sequences disclosed herein. These techniques are standard and well-established (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology*, 3rd Edition, pages 4-1 to 4-6 (John Wiley & Sons 1995) ("Ausubel (1995)"); Wu *et al.*, *Methods in Gene Biotechnology*, pages 33-41 (CRC Press, Inc. 1997) ("Wu (1997)"); Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327)).

Alternatively, molecules for constructing immunoglobulin fusion proteins can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol.* 21:1131 (1993), Bambot *et al.*, *PCR Methods and Applications* 2:266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al.*, *PCR Methods Appl.* 4:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al.*, *Annu. Rev. Biochem.* 53:323 (1984), and Climie *et al.*, *Proc. Nat'l Acad. Sci. USA* 87:633 (1990).

4. Production of TACI-Immunoglobulin Polypeptides

The polypeptides of the present invention can be produced in recombinant host cells following conventional techniques. To express a TACI-immunoglobulin-encoding sequence, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene, which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial

replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence.

Expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, an expression vector may comprise a nucleotide sequence that encodes TACI-immunoglobulin and a secretory sequence derived from any secreted gene. As discussed above, one suitable signal sequence is a tPA signal sequence. An exemplary tPA signal sequence is provided by SEQ ID NO:25. Another suitable signal sequence is a murine 26-10 V_H signal sequence. The murine 26-10 antibody is described, for example, by Near *et al.*, *Mol. Immunol.* 27:901 (1990). Illustrative amino acid and nucleotide sequences of a murine 26-10 V_H signal sequence are provided by SEQ ID NO:61 and SEQ ID NO:65, respectively. SEQ ID NO:62 discloses the amino acid sequence of a TACI-Fc5 fusion protein that comprises a murine 26-10 V_H signal sequence.

TACI-immunoglobulin proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al.*, *J. Molec. Appl. Genet.* 1:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell* 31:355 (1982)), the

SV40 early promoter (Benoist *et al.*, *Nature* 290:304 (1981)), the Rous sarcoma virus promoter (Gorman *et al.*, *Proc. Nat'l Acad. Sci. USA* 79:6777 (1982)), the cytomegalovirus promoter (Foecking *et al.*, *Gene* 45:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)). One useful combination of a promoter and enhancer is provided by a myeloproliferative sarcoma virus promoter and a human cytomegalovirus enhancer.

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control production of TACI-immunoglobulin proteins in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al.*, *Mol. Cell. Biol.* 10:4529 (1990), and Kaufman *et al.*, *Nucl. Acids Res.* 19:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel (1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

TACI-immunoglobulin polypeptides can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al.*, *Meth. Cell Biol.* 43:161 (1994), and Douglas and Curiel, *Science & Medicine* 4:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al.*, *Cytotechnol.* 15:145 (1994)).

Those of skill in the art can devise suitable expression vectors for producing the fusion proteins described herein with mammalian cells. Example 4 describes features of one expression vector. As another example, an expression vector can comprise a bicistronic expression cassette that includes a portion of the human cytomegalovirus enhancer, the myeloproliferative sarcoma virus promoter, a nucleotide sequence encoding a fusion protein, the poliovirus internal ribosomal entry sites, a nucleotide sequence encoding murine dihydrofolate reductase, followed by the SV40 poly A addition sequence. The nucleotide sequence of SEQ ID NO:69 shows a cytomegalovirus enhancer/myeloproliferative sarcoma virus LTR promoter construct, in which the cytomegalovirus enhancer extends from nucleotide 1 to 407. The myeloproliferative sarcoma virus LTR promoter, absent the negative control region extends from nucleotide 408 to nucleotide 884 of SEQ ID NO:69. A nucleotide sequence for the myeloproliferative sarcoma virus LTR promoter without the negative control region is provided in SEQ ID NO:70.

Example 1 describes an expression vector that comprises a cytomegalovirus promoter to direct the expression of the recombinant protein transgene, an immunoglobulin intron, and a tissue plasminogen activator signal sequence. One suitable immunoglobulin intron is a murine 26-10 V_H intron. SEQ ID NO:66 provides an illustrative nucleotide sequence of a murine 26-10 V_H intron. An expression vector

may also include a 5' untranslated region (UTR) located upstream of the nucleotide sequence that encodes a TACI-immunoglobulin protein. A suitable 5'-UTR can be derived from the murine 26-10 V_H gene. SEQ ID NO:63 discloses the nucleotide sequence of a useful native murine 26-10 V_H 5'-UTR, while SEQ ID NO:64 shows the nucleotide sequence of a murine 26-10 V_H 5'-UTR, which has been optimized at the 3' end.

As an illustration, SEQ ID NO:67 provides a nucleotide sequence that includes the following elements: a native murine 26-10 V_H 5'-UTR (nucleotides 1 to 51), a murine 26-10 V_H signal sequence (nucleotides 52 to 97, and 182 to 192), a murine 26-10 V_H intron (nucleotides 98 to 181), a nucleotide sequence that encodes a TACI moiety (nucleotides 193 to 435), and a nucleotide sequence that encodes an Fc5 moiety (nucleotides 436 to 1131). The nucleotide sequence of SEQ ID NO:68 differs from SEQ ID NO:67 due to the replacement of an optimized murine 26-10 V_H 5'-UTR (nucleotides 1 to 51) for the native sequence.

TACI-immunoglobulin proteins can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned genes into insect cells. Suitable expression vectors are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein (hsp)* 70 promoter, *Autographa californica nuclear polyhedrosis virus immediate-early* gene promoter (*ie-1*) and the *delayed early 39K* promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al.*, *J. Virol.* 67:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the TACI-immunoglobulin polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol.* 71:971 (1990), Bonning, *et al.*, *J. Gen. Virol.* 75:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem.* 270:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed TACI-immunoglobulin polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al.*, *Proc. Nat'l Acad. Sci.* 82:7952 (1985)). Using a technique known in the art, a transfer vector containing a nucleotide sequence that encodes a TACI-immunoglobulin protein is transformed into *E. coli*, and screened for bacmids, which contain an interrupted *lacZ* gene indicative of recombinant baculovirus.

The bacmid DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as *Pcor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol.* 71:971 (1990), Bonning, *et al.*, *J. Gen. Virol.* 75:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed, with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen; San Diego, CA) can be used in such constructs.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-Sf-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as Sf9 (ATCC CRL 1711), Sf21AE, and Sf21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH FIVEO cell line (Invitrogen) derived from *Trichoplusia ni*, (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cello405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately $2-5 \times 10^5$ cells to a density of $1-2 \times 10^6$ cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.*, "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression Protocols*, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GAL1* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOX1* (alcohol oxidase), *HIS4* (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. A vector can be designed to generate constructs utilizing the necessary elements to carry out homologous recombination in yeast (see, for example, Raymond *et al.*, *BioTechniques* 26:134 (1999)). For example, such an expression vector can include URA3 and CEN-ARS (autonomously replicating sequence) sequences required for selection and replication in *S. cerevisiae*. Other suitable vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YEp vectors such as YEp13 and YCp vectors, such as YCp19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides from these cells are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311, Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al.*, *Yeast* 14:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565.

5 DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the
10 dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica* *ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase
15 (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the
20 introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (*t*) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

25 Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al.*, *Science* 227:1229 (1985), Klein *et al.*, *Biotechnology* 10:268 (1992), and Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).
30

Alternatively, TACI-immunoglobulin proteins can be produced in prokaryotic host cells. Suitable promoters that can be used to produce TACI-immunoglobulin polypeptides in a prokaryotic host are well-known to those of skill in
35 the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the *P_R* and *P_L* promoters of bacteriophage lambda, the *trp*, *recA*, heat

shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli*, promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been
5 reviewed by Glick, *J. Ind. Microbiol.* 1:277 (1987), Watson *et al.*, *Molecular Biology of the Gene*, 4th Ed. (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101,
10 JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilis* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

15 When expressing a TACI-immunoglobulin protein in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be
20 refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic
25 space and recovering the protein, thereby obviating the need for denaturation and refolding.

Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in
30 *DNA Cloning 2: Expression Systems*, 2nd Edition, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley &
35 Sons, Inc. 1996)).

Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, 5 Grishammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* 10 (The Humana Press, Inc. 1995).

As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc.* 85:2149 (1963), Stewart *et al.*, 15 "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot.* 3:3 (1986), Atherton *et al.*, *Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al.*, *Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC 20 Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al.*, *Science* 266:776 (1994), Hackeng *et al.*, *Proc. Nat'l Acad. Sci. USA* 94:7845 (1997), Dawson, *Methods Enzymol.* 287: 34 (1997), Muir *et al.*, *Proc. Nat'l Acad. Sci. USA* 95:6705 (1998), and Severinov and Muir, *J. Biol. Chem.* 273:16205 25 (1998)).

5. Assays for TACI-Immunoglobulin Fusion Proteins

The function of TACI-immunoglobulin fusion proteins can be examined using a variety of approaches to assess the ability of the fusion proteins to bind ZTNF4 or ZTNF2. As an illustration, Example 4 provides methods for measuring ZTNF4 30 binding affinity and binding capacity.

Alternatively, TACI-immunoglobulin fusion proteins can be characterized by the ability to inhibit the stimulation of human B cells by soluble ZTNF4, as described by Gross *et al.*, international publication No. WO00/40716. Briefly, human B cells are 35 isolated from peripheral blood mononuclear cells using CD19 magnetic beads and the VarioMacs magnetic separation system (Miltenyi Biotec Auburn, CA) according to the

manufacturer's instructions. Purified B cells are mixed with soluble ZTNF4 (25 ng/ml) and recombinant human IL-4 (10 ng/ml Pharmingen), and the cells are plated onto round bottom 96 well plates at 1×10^5 cells per well.

5 Soluble TACI-immunoglobulin proteins can be diluted from about 5 μ g/ml to about 6 ng/ml, and incubated with the B cells for five days, pulsing overnight on day four with 1 μ Ci 3 H-thymidine per well. As a control, TACI-immunoglobulin protein can also be incubated with B cells and IL-4 without ZTNF4. Plates are harvested using Packard plate harvester, and counted using the Packard reader.

10 This general approach was used to examine three TACI-Fc fusion proteins. Although all fusion proteins inhibited B cell proliferation, constructs TACI (d1-29, d111-154)-Fc5 and TACI (d1-29, d120-154)-Fc5 were more potent than TACI (d1-29, d107-154)-Fc5.

15 Well-established animal models are available to test *in vivo* efficacy of TACI-immunoglobulin proteins in certain disease states. For example, TACI-immunoglobulin proteins can be tested in a number of animal models of autoimmune disease, such as MRL-*lpr/lpr* or NZB x NZW F1 congenic mouse strains, which serve as a model of SLE (systemic lupus erythematosus). Such animal models are known in the art (see, for example, Cohen and Miller (Eds.), *Autoimmune Disease Models: A Guidebook* (Academic Press, Inc. 1994).

20 Offspring of a cross between New Zealand Black (NZB) and New Zealand White (NZW) mice develop a spontaneous form of SLE that closely resembles SLE in humans. The offspring mice, known as NZBW begin to develop IgM autoantibodies against T-cells at one month of age, and by five to seven months of age, anti-DNA autoantibodies are the dominant immunoglobulin. Polyclonal B-cell
25 hyperactivity leads to overproduction of autoantibodies. The deposition of these autoantibodies, particularly those directed against single stranded DNA, is associated with the development of glomerulonephritis, which manifests clinically as proteinuria, azotemia, and death from renal failure.

30 Kidney failure is the leading cause of death in mice affected with spontaneous SLE, and in the NZBW strain, this process is chronic and obliterative. The disease is more rapid and severe in females than males, with mean survival of only 245 days as compared to 406 days for the males. While many of the female mice will be symptomatic (proteinuria) by seven to nine months of age, some can be much younger or older when they develop symptoms. The fatal immune nephritis seen in the NZBW mice
35 is very similar to the glomerulonephritis seen in human SLE, making this spontaneous murine model very attractive for testing of potential SLE therapeutics (Putterman and Naparstek, "Murine Models of Spontaneous Systemic Lupus Erythematosus," *in*

Autoimmune Disease Models: A Guidebook, pages 217-234 (Academic Press, Inc., 1994); Mohan *et al.*, *J. Immunol.* 154:1470 (1995); and Daikh *et al.*, *J. Immunol.* 159:3104 (1997)).

As described by Gross *et al.*, international publication No. WO00/40716, TACI-immunoglobulin proteins can be administered to NZBW mice to monitor its suppressive effect on B cells over the five-week period when, on average, B-cell autoantibody production is believed to be at high levels in NZBW mice. Briefly, 100 8-week old female (NZB x NZW)_{F1} mice can be divided into six groups of 15 mice. Prior to treatment, the mice are monitored once a month for urine protein, and blood is drawn for CBC and serum banking. Serum can be screened for the presence of autoantibodies. Because proteinuria is the hallmark sign of glomerulonephritis, urine protein levels are monitored by dipstick at regular intervals over the course of the study. Treatment can begin when mice are approximately five months of age. The mice receive intraperitoneal injections of vehicle only (phosphate buffered saline) or human TACI-immunoglobulin (control protein) or TACI-immunoglobulin protein (*e.g.*, 20 to 100 µg test protein per dose) three times a week for five weeks.

Blood is collected twice during treatment, and will be collected at least twice following treatment. Urine dipstick values for proteinuria and body weights are determined every two weeks after treatment begins. Blood, urine dipstick value and body weight are collected at the time of euthanasia. The spleen and thymus are divided for fluorescent activated cell sorting analysis and histology. Submandibular salivary glands, mesenteric lymph node chain, liver lobe with gall bladder, cecum and large intestine, stomach, small intestine, pancreas, right kidney, adrenal gland, tongue with trachea and esophagus, heart and lungs are also collected for histology.

Murine models for experimental allergic encephalomyelitis have been used as a tool to investigate both the mechanisms of immune-mediated disease, and methods of potential therapeutic intervention. The model resembles human multiple sclerosis, and produces demyelination as a result of T-cell activation to neuroproteins such as myelin basic protein, or proteolipid protein. Inoculation with antigen leads to induction of CD4+, class II MHC-restricted T-cells (Th1). Changes in the protocol for experimental allergic encephalomyelitis can produce acute, chronic-relapsing, or passive-transfer variants of the model (Weinberg *et al.*, *J. Immunol.* 162:1818 (1999); Mijaba *et al.*, *Cell. Immunol.* 186:94 (1999); and Glabinski, *Meth. Enzym.* 288:182 (1997)).

Gross *et al.*, international publication No. WO00/40716, describe one approach to evaluating the efficacy of TACI-immunoglobulin proteins in the amelioration of symptoms associated with experimental allergic encephalomyelitis. Briefly, 25 female PLxSJL F1 mice (12 weeks old) are given a subcutaneous injection of

125 µg/mouse of antigen (myelin Proteolipid Protein, PLP, residues 139-151), formulated in complete Freund's Adjuvant. The mice are divided into five groups of five mice. Intraperitoneal injections of pertussis toxin (400 ng) are given on Day 0 and 2. The groups are given a 1x, 10x, or 100x dose of TACI-immunoglobulin protein, one group will receive vehicle only, and one group will receive no treatment. Prevention therapy begins on Day 0, intervention therapy begins on day 7, or at onset of clinical signs. Signs of disease, weight loss, and paralysis manifest in approximately 10 to 14 days, and last for about one week. Animals are assessed daily by collecting body weights and assigning a clinical score to correspond to the extent of their symptoms. Clinical signs of experimental allergic encephalomyelitis appear within 10 to 14 days of inoculation and persist for approximately one week. At the end of the study, all animals are euthanized by gas overdose, and necropsied. The brain and spinal column are collected for histology or frozen for mRNA analysis. Body weight and clinical score data are plotted by individual and by group.

In the collagen-induced arthritis model, mice develop chronic inflammatory arthritis, which closely resembles human rheumatoid arthritis. Since collagen-induced arthritis shares similar immunological and pathological features with rheumatoid arthritis, this makes it an ideal model for screening potential human anti-inflammatory compounds. Another advantage in using the collagen-induced arthritis model is that the mechanisms of pathogenesis are known. The T and B cell epitopes on type II collagen have been identified, and various immunological (delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediating arthritis have been determined, and can be used to assess test compound efficacy in the models (Wooley, *Curr. Opin. Rheum.* 3:407 (1999); Williams *et al.*, *Immunol.* 89:9784 (1992); Myers *et al.*, *Life Sci.* 61:1861 (1997); and Wang *et al.*, *Immunol.* 92:8955 (1995)).

Gross *et al.*, international publication No. WO00/40716, describe a method for evaluating the efficacy of TACI-immunoglobulin proteins in the amelioration of symptoms associated with collagen-induced arthritis. In brief, eight-week old male DBA/1J mice (Jackson Labs) are divided into groups of five mice/group and are given two subcutaneous injections of 50 to 100 µl of 1 mg/ml collagen (chick or bovine origin), at three week intervals. One control does not receive collagen injections. The first injection is formulated in Complete Freund's Adjuvant, and the second injection is formulated in Incomplete Freund's Adjuvant. TACI-immunoglobulin protein is administered prophylactically at or before the second injection, or after the animal develops a clinical score of two or more that persists at least 24 hours. Animals begin to show symptoms of arthritis following the second collagen injection, usually within two

to three weeks. For example, TACI-Fc, a control protein, human IgFc, or phosphate-buffered saline (vehicle) can be administered prophylactically beginning seven days before the second injection (day -7). Proteins can be administered at 100 µg, given three times a week as a 200 µl intraperitoneal injection, and continued for four weeks.

5 In the collagen-induced arthritis model, the extent of disease is evaluated in each paw using a caliper to measure paw thickness and assigning a clinical score to each paw. For example, a clinical score of "0" indicates a normal mouse, a score of "1" indicates that one or more toes are inflamed, a score of "2" indicates mild paw inflammation, a score of "3" indicates moderate paw inflammation, and a score of "4" indicates severe paw inflammation. Animals are euthanized after the disease as been
10 established for a set period of time, usually seven days. Paws are collected for histology or mRNA analysis, and serum is collected for immunoglobulin and cytokine assays.

 Myasthenia gravis is another autoimmune disease for which murine models are available. Myasthenia gravis is a disorder of neuromuscular transmission
15 involving the production of autoantibodies directed against the nicotinic acetylcholine receptor. This disease is acquired or inherited with clinical features including abnormal weakness and fatigue on exertion.

 A murine model of myasthenia gravis has been established. (Christadoss
20 *et al.*, "Establishment of a Mouse Model of Myasthenia gravis Which Mimics Human Myasthenia gravis Pathogenesis for Immune Intervention," in *Immunobiology of Proteins and Peptides VIII*, Atassi and Bixler (Eds.), pages 195-199 (1995)). Experimental autoimmune myasthenia gravis is an antibody mediated disease characterized by the presence of antibodies to acetylcholine receptor. These antibodies destroy the receptor leading to defective neuromuscular electrical impulses, resulting in
25 muscle weakness. In the experimental autoimmune myasthenia gravis model, mice are immunized with the nicotinic acetylcholine receptor. Clinical signs of myasthenia gravis become evident weeks after the second immunization. Experimental autoimmune myasthenia gravis is evaluated by several methods including measuring serum levels of acetylcholine receptor antibodies by radioimmunoassay (Christadoss and Dauphinee, *J. Immunol.* 136:2437 (1986); Lindstrom *et al.*, *Methods Enzymol.* 74:432 (1981)),
30 measuring muscle acetylcholine receptor, or electromyography (Coligan *et al.* (Eds.), *Protocols in Immunology. Vol.3*, page 15.8.1 (John Wiley & Sons, 1997)).

 The effect of TACI-immunoglobulin on experimental autoimmune myasthenia gravis can be determined by administering fusion proteins during ongoing
35 clinical myasthenia gravis in B6 mice. For example, 100 B6 mice are immunized with 20 µg acetylcholine receptor in complete Freund's adjuvant on days 0 and 30. Approximately 40 to 60% of mice will develop moderate (grade 2) to severe (grade 3)

clinical myasthenia gravis after the boost with acetylcholine receptor. Mice with grade 2 and 3 clinical disease are divided into three groups (with equal grades of weakness) and weighed (mice with weakness also lose weight, since they have difficulty in consuming food and water) and bled for serum (for pre-treatment anti-acetylcholine receptor antibody and isotype level). Group A is injected I.P with phosphate buffered saline, group B is injected intraperitoneally with human IgG-Fc as a control protein (100 µg), and group C is injected with 100 µg of TACI-Fc three times a week for four weeks. Mice are screened for clinical muscle weakness twice a week, and weighed and bled for serum 15 and 30 days after the commencement of treatment. Whole blood is collected on day 15 to determine T/B cell ratio by fluorescence activated cell sorter analysis using markers B220 and CD5. Surviving mice are killed 30 to 45 days after the initiation of treatment, and their carcasses are frozen for later extraction of muscle acetylcholine receptor to determine the loss of muscle acetylcholine receptor, the primary pathology in myasthenia gravis (see, for example, Coligan *et al.* (Eds.), *Protocols in Immunology*. Vol. 3, page 15.8.1 (John Wiley & Sons, 1997)).

Serum antibodies to mouse muscle acetylcholine receptor can be determined by an established radioimmunoassay, and anti-acetylcholine receptor antibody isotypes (IgM, IgG1, IgG2b and IgG2c) is measured by ELISA. Such methods are known. The effects of TACI-immunoglobulin on ongoing clinical myasthenia gravis, anti-acetylcholine receptor antibody and isotype level, and muscle acetylcholine receptor loss are determined.

Approximately 100 mice can be immunized with 20 µg acetylcholine receptor in complete Freund's adjuvant on day 0 and 30. Mice with clinical myasthenia gravis are divided into four groups. Group A is injected intraperitoneally with 100 µg control Fc, group B is injected with 20 µg control Fc, group C is injected with 100 µg TACI-Fc, and group D is injected with 20 µg TACI-Fc three times a week for four weeks. Mice are weighed and bled for serum before, and 15 and 30 days after the start of the treatment. Serum is tested for anti-acetylcholine receptor antibody and isotypes as described above. Muscle acetylcholine receptor loss can also be measured.

Other suitable assays of TACI-immunoglobulin fusion proteins can be determined by those of skill in the art.

6. *Production of TACI-Immunoglobulin Conjugates*

The present invention includes chemically modified TACI-immunoglobulin compositions, in which a TACI-immunoglobulin polypeptide is linked with a polymer. Typically, the polymer is water-soluble so that the TACI-

immunoglobulin conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation. In this way, the degree of polymerization can be controlled. An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C₁-C₁₀) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce TACI-immunoglobulin conjugates.

TACI-immunoglobulin conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include polyethylene glycol (PEG), monomethoxy-PEG, mono-(C₁-C₁₀)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A TACI-immunoglobulin conjugate can also comprise a mixture of such water-soluble polymers.

One example of a TACI-immunoglobulin conjugate comprises a TACI-immunoglobulin moiety and a polyalkyl oxide moiety attached to the *N*-terminus of the TACI-immunoglobulin. PEG is one suitable polyalkyl oxide. As an illustration, TACI-immunoglobulin can be modified with PEG, a process known as "PEGylation." PEGylation of TACI-immunoglobulin can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems* 9:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet.* 27:290 (1994), and Francis *et al.*, *Int J Hematol* 68:1 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, TACI-immunoglobulin conjugates are formed by condensing activated PEG, in which a terminal hydroxy or amino group of PEG has been replaced by an activated linker (see, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

PEGylation by acylation typically requires reacting an active ester derivative of PEG with a TACI-immunoglobulin polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between TACI-immunoglobulin and a water-soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated TACI-immunoglobulin by acylation will typically

comprise the steps of (a) reacting a TACI-immunoglobulin polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to TACI-immunoglobulin, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:TACI-immunoglobulin, the greater the percentage of polyPEGylated TACI-immunoglobulin product.

The product of PEGylation by acylation is typically a polyPEGylated TACI-immunoglobulin product, wherein the lysine ϵ -amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting TACI-immunoglobulin will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated TACI-immunoglobulin polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with TACI-immunoglobulin in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a $-\text{CH}_2\text{-NH}$ group.

Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the ϵ -amino groups of the lysine residues and the α -amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of TACI-immunoglobulin monopolymer conjugates.

Reductive alkylation to produce a substantially homogenous population of monopolymer TACI-immunoglobulin conjugate molecule can comprise the steps of: (a) reacting a TACI-immunoglobulin polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the α -amino group at the amino terminus of the TACI-immunoglobulin, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the Schiff base formed in the initial process of reductive

alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

For a substantially homogenous population of monopolymer TACI-immunoglobulin conjugates, the reductive alkylation reaction conditions are those that permit the selective attachment of the water soluble polymer moiety to the *N*-terminus of TACI-immunoglobulin. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the α -amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal α -group, the more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:TACI-immunoglobulin need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6.

Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to TACI-immunoglobulin will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to TACI-immunoglobulin will be 1:1 to 20:1 for polyPEGylation, and 1:1 to 5:1 for monoPEGylation.

General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al.*, *Clin. Pharmacol. Ther.* 59:636 (1996), Monkarsh *et al.*, *Anal. Biochem.* 247:434 (1997)).

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

7. Isolation of TACI-Immunoglobulin Polypeptides

The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the

present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Fractionation and/or conventional purification methods can be used to obtain preparations of synthetic TACI-immunoglobulin polypeptides, and recombinant TACI-immunoglobulin polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988), and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

Additional variations in TACI-immunoglobulin isolation and purification can be devised by those of skill in the art. For example, anti-TACI or anti-Fc antibodies can be used to isolate large quantities of protein by immunoaffinity purification.

The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem.* 3:1 (1985)). Histidine-rich proteins will

be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography, Protein A chromatography, and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol.* 182:529 (1990)).

TACI-immunoglobulin polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. TACI-immunoglobulin polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue. A TACI-immunoglobulin fusion protein may be non-glycosylated, glycosylated, or glycosylated only in the TACI moiety or in the immunoglobulin moiety. The immunoglobulin moiety can be obtained from a human antibody, a chimeric antibody, or a humanized antibody.

8. Therapeutic Uses of TACI-Immunoglobulin Polypeptides

TACI-immunoglobulin proteins can be used to modulate the immune system by binding ZTNF4 or ZTNF2, and thus, preventing the binding of these ligands with endogenous TACI or BCMA receptors. Accordingly, the present invention includes the use of TACI-immunoglobulin proteins to a subject, which lacks an adequate amount of TACI or BCMA receptors, or which produces an excess of ZTNF4 or ZTNF2. These molecules can be administered to any subject in need of treatment, and the present invention contemplates both veterinary and human therapeutic uses. Illustrative subjects include mammalian subjects, such as farm animals, domestic animals, and human patients.

TACI-immunoglobulin polypeptides can be used for the treatment of autoimmune diseases, B cell cancers, immunomodulation, IBD and any antibody-mediated pathologies (*e.g.*, ITCP, myasthenia gravis and the like), renal diseases, indirect T cell immune response, graft rejection, and graft versus host disease. The polypeptides of the present invention can be targeted to specifically regulate B cell responses during the immune response. Additionally, the polypeptides of the present invention can be used to modulate B cell development, development of other cells, antibody production, and cytokine production. Polypeptides of the present invention can also modulate T and B cell communication by neutralizing the proliferative effects of ZTNF4.

TACI-immunoglobulin polypeptides of the present invention can be useful to neutralize the effects of ZTNF4 for treating pre-B or B-cell leukemias, such as plasma cell leukemia, chronic or acute lymphocytic leukemia, myelomas such as

multiple myeloma, plasma cell myeloma, endothelial myeloma and giant cell myeloma, and lymphomas such as non-Hodgkins lymphoma, for which an increase in ZTNF4 polypeptides is associated.

5 ZTNF4 is expressed in CD8⁺ cells, monocytes, dendritic cells, activated monocytes, which indicates that, in certain autoimmune disorders, cytotoxic T-cells might stimulate B-cell production through excess production of ZTNF4. Immunosuppressant proteins that selectively block the action of B-lymphocytes would be of use in treating disease. Autoantibody production is common to several autoimmune diseases and contributes to tissue destruction and exacerbation of disease.
10 Autoantibodies can also lead to the occurrence of immune complex deposition complications and lead to many symptoms of systemic lupus erythematosus, including kidney failure, neuralgic symptoms and death. Modulating antibody production independent of cellular response would also be beneficial in many disease states. B cells have also been shown to play a role in the secretion of arthritogenic immunoglobulins in
15 rheumatoid arthritis. As such, inhibition of ZTNF4 antibody production would be beneficial in treatment of autoimmune diseases such as myasthenia gravis, rheumatoid arthritis, polyarticular-course juvenile rheumatoid arthritis, and psoriatic arthritis. Immunosuppressant therapeutics such as TACI-immunoglobulin proteins that selectively block or neutralize the action of B-lymphocytes would be useful for such purposes.

20 The invention provides methods employing TACI-immunoglobulin proteins for selectively blocking or neutralizing the actions of B-cells in association with end stage renal diseases, which may or may not be associated with autoimmune diseases. Such methods would also be useful for treating immunologic renal diseases. Such methods would be useful for treating glomerulonephritis associated with
25 diseases such as membranous nephropathy, IgA nephropathy or Berger's Disease, IgM nephropathy, Goodpasture's Disease, post-infectious glomerulonephritis, mesangioproliferative disease, chronic lymphoid leukemia, minimal-change nephrotic syndrome. Such methods would also serve as therapeutic applications for treating secondary glomerulonephritis or vasculitis associated with such diseases as lupus,
30 polyarteritis, Henoch-Schonlein, Scleroderma, HIV-related diseases, amyloidosis or hemolytic uremic syndrome. The methods of the present invention would also be useful as part of a therapeutic application for treating interstitial nephritis or pyelonephritis associated with chronic pyelonephritis, analgesic abuse, nephrocalcinosis, nephropathy caused by other agents, nephrolithiasis, or chronic or acute interstitial nephritis.

35 The methods of the present invention also include use of TACI-immunoglobulin proteins in the treatment of hypertensive or large vessel diseases, including renal artery stenosis or occlusion and cholesterol emboli or renal emboli.

The present invention also provides methods for treatment of renal or urological neoplasms, multiple myelomas, lymphomas, light chain neuropathy or amyloidosis.

5 The invention also provides methods for blocking or inhibiting activated B cells using TACI-immunoglobulin proteins for the treatment of asthma and other chronic airway diseases such as bronchitis and emphysema. The TACI-immunoglobulin proteins described herein can also be used to treat Sjögren's Syndrome.

Also provided are methods for inhibiting or neutralizing an effector T cell response using TACI-immunoglobulin proteins for use in immunosuppression, in particular for such therapeutic use as for graft-versus-host disease and graft rejection. 10 Moreover, TACI-immunoglobulin proteins would be useful in therapeutic protocols for treatment of such autoimmune diseases as insulin dependent diabetes mellitus (IDDM) and Crohn's Disease. Methods of the present invention would have additional therapeutic value for treating chronic inflammatory diseases, in particular to lessen joint pain, swelling, anemia and other associated symptoms as well as treating septic shock. 15

Well established animal models are available to test *in vivo* efficacy of TACI-immunoglobulin proteins of the present invention in certain disease states. In particular, TACI-immunoglobulin proteins can be tested *in vivo* in a number of animal models of autoimmune disease, such as MRL-*lpr/lpr* or NZB x NZW F1 congenic mouse strains which serve as a model of SLE (systemic lupus erythematosus). Such animal 20 models are known in the art.

Offspring of a cross between New Zealand Black (NZB) and New Zealand White (NZW) mice develop a spontaneous form of SLE that closely resembles SLE in humans. The offspring mice, known as NZBW begin to develop IgM autoantibodies against T-cells at 1 month of age, and by 5-7 months of age, Ig anti-DNA autoantibodies are the dominant immunoglobulin. Polyclonal B-cell hyperactivity leads to overproduction of autoantibodies. The deposition of these autoantibodies, particularly ones directed against single stranded DNA is associated with the development of glomerulonephritis, which manifests clinically as proteinuria, azotemia, and death from renal failure. Kidney failure is the leading cause of death in mice affected with spontaneous SLE, and in the NZBW strain, this process is chronic and obliterative. The disease is more rapid and severe in females than males, with mean survival of only 245 days as compared to 406 days for the males. While many of the female mice will be symptomatic (proteinuria) by 7-9 months of age, some can be much younger or older when they develop symptoms. The fatal immune nephritis seen in the NZBW mice is very similar to the glomerulonephritis seen in human SLE, making this spontaneous 35 murine model useful for testing of potential SLE therapeutics.

Mouse models for experimental allergic encephalomyelitis (EAE) has been used as a tool to investigate both the mechanisms of immune-mediated disease, and methods of potential therapeutic intervention. The model resembles human multiple sclerosis, and produces demyelination as a result of T-cell activation to neuroproteins such as myelin basic protein (MBP), or proteolipid protein (PLP). Inoculation with antigen leads to induction of CD4+, class II MHC-restricted T-cells (Th1). Changes in the protocol for EAE can produce acute, chronic-relapsing, or passive-transfer variants of the model.

In the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis, which closely resembles human rheumatoid arthritis (RA). Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. Another advantage in using the CIA model is that the mechanisms of pathogenesis are known. The T and B cell epitopes on type II collagen have been identified, and various immunological (delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediating arthritis have been determined, and can be used to assess test compound efficacy in the models.

Myasthenia gravis (MG) is another autoimmune disease for which murine models are available. MG is a disorder of neuromuscular transmission involving the production of autoantibodies directed against the nicotinic acetylcholine receptor (AChR). MG is acquired or inherited with clinical features including abnormal weakness and fatigue on exertion. A mouse model of MG has been established. Experimental autoimmune myasthenia gravis (EAMG) is an antibody mediated disease characterized by the presence of antibodies to AChR. These antibodies destroy the receptor leading to defective neuromuscular electrical impulses, resulting in muscle weakness. In the EAMG model, mice are immunized with the nicotinic acetylcholine receptor. Clinical signs of MG become evident weeks after the second immunization. EAMG is evaluated by several methods including measuring serum levels of AChR antibodies by radioimmunoassay, measuring muscle AChR, or electromyography.

Generally, the dosage of administered TACI-immunoglobulin protein will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of TACI-immunoglobulin protein, which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of subject), although a lower or higher dosage also may be administered as circumstances dictate.

Administration of a TACI-immunoglobulin protein to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev.* 35:199 (1999)). Dry or liquid particles comprising TACI-immunoglobulin can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (e.g., Pettit and Gombotz, *TIBTECH* 16:343 (1998); Patton *et al.*, *Adv. Drug Deliv. Rev.* 35:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of transcutaneous administration (Mitragotri *et al.*, *Science* 269:850 (1995)). Transdermal delivery using electroporation provides another means to administer a TACI-immunoglobulin protein (Potts *et al.*, *Pharm. Biotechnol.* 10:213 (1997)).

A pharmaceutical composition comprising a TACI-immunoglobulin protein can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, TACI-immunoglobulin proteins are administered to a patient in a therapeutically effective amount. A TACI-immunoglobulin protein and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology

of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor. Furthermore, an agent used to treat systemic lupus erythematosus is physiologically significant if the administration of the agent results in a decrease of circulating anti-double stranded DNA antibodies, or a decrease in at least one of the following symptoms: fever, joint pain, erythematosus skin lesions, or other features of systemic lupus erythematosus. One example of a general indication that a TACI-immunoglobulin protein is administered in a therapeutically effective amount is that, following administration to a subject, there is a decrease in circulating levels of ZTNF4 (BLyS).

A pharmaceutical composition comprising a TACI-immunoglobulin protein can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al.*, *Pharm. Biotechnol.* 10:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)).

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1):S61 (1993), Kim, *Drugs* 46:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02 μm to greater than 10 μm . A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous

space(s) (see, for example, Machy *et al.*, *Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al.*, *American J. Hosp. Pharm.* 46:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al.*, *Ann. N.Y. Acad. Sci.* 446:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al.*, *Biochim. Biophys. Acta* 802:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al.*, *Biochim. Biophys. Acta* 1068:133 (1991); Allen *et al.*, *Biochim. Biophys. Acta* 1150:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al.*, *Biol. Pharm. Bull.* 16:960 (1993)). These formulations were prepared by mixing soybean phosphatidylcholine, α -tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al.*, *Biol. Pharm. Bull.* 20:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosylipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug*

Carrier Syst. 14:287 (1997); Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Similarly, Wu *et al.*, *Hepatology* 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Polyacetylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al.*, *Proc. Nat'l Acad. Sci. USA* 94:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)).

TACI-immunoglobulin proteins can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al.*, *Infect. Immun.* 31:1099 (1981), Anderson *et al.*, *Cancer Res.* 50:1853 (1990), and Cohen *et al.*, *Biochim. Biophys. Acta* 1063:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology*, 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al.*, *Meth. Enzymol.* 149:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al.*, *Biochim. Biophys. Acta* 1150:9 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly(ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem.* 6:332 (1995); Ranade, "Role of Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al.*, *Science* 281:1161 (1998); Putney and Burke, *Nature Biotechnology* 16:153 (1998); Putney, *Curr. Opin. Chem. Biol.* 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can

also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al.*, *Pharm. Biotechnol.* 10:167 (1997)).

The present invention also contemplates chemically modified TACI-immunoglobulin proteins in which the polypeptide is linked with a polymer, as discussed
5 above.

Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger,
10 *Drug Delivery Systems* (CRC Press 1996).

As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a TACI-immunoglobulin protein. Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively,
15 such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the TACI-immunoglobulin protein composition is contraindicated in patients with known hypersensitivity to either the
20 TACI receptor moiety or the immunoglobulin moiety.

9. Therapeutic Uses of TACI-Immunoglobulin Nucleotide Sequences

The present invention includes the use of nucleic acid molecules that encode TACI-immunoglobulin fusion proteins to provide these fusion proteins to a
25 subject in need of such treatment. For veterinary therapeutic use or human therapeutic use, such nucleic acid molecules can be administered to a subject having a disorder or disease, as discussed above. As one example discussed earlier, nucleic acid molecules encoding a TACI-immunoglobulin fusion protein can be used for long-term treatment of systemic lupus erythematosus.

There are numerous approaches for introducing a *TACI-immunoglobulin* gene to a subject; including the use of recombinant host cells that express *TACI-immunoglobulin*, delivery of naked nucleic acid encoding TACI-immunoglobulin, use of a cationic lipid carrier with a nucleic acid molecule that encodes TACI-immunoglobulin, and the use of viruses that express *TACI-immunoglobulin*, such as recombinant
35 retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses (see, for example, Mulligan, *Science* 260:926

(1993), Rosenberg *et al.*, *Science* 242:1575 (1988), LaSalle *et al.*, *Science* 259:988 (1993), Wolff *et al.*, *Science* 247:1465 (1990), Breakfield and Deluca, *The New Biologist* 3:203 (1991)). In an *ex vivo* approach, for example, cells are isolated from a subject, transfected with a vector that expresses a *TACI-immunoglobulin* gene, and then transplanted into the subject.

In order to effect expression of a *TACI-immunoglobulin* gene, an expression vector is constructed in which a nucleotide sequence encoding a *TACI-immunoglobulin* gene is operably linked to a core promoter, and optionally a regulatory element, to control gene transcription. The general requirements of an expression vector are described above.

Alternatively, a *TACI-immunoglobulin* gene can be delivered using recombinant viral vectors, including for example, adenoviral vectors (*e.g.*, Kass-Eisler *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:11498 (1993), Kolls *et al.*, *Proc. Nat'l Acad. Sci. USA* 91:215 (1994), Li *et al.*, *Hum. Gene Ther.* 4:403 (1993), Vincent *et al.*, *Nat. Genet.* 5:130 (1993), and Zabner *et al.*, *Cell* 75:207 (1993)), adenovirus-associated viral vectors (Flotte *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:10613 (1993)), alphaviruses such as Semliki Forest Virus and Sindbis Virus (Hertz and Huang, *J. Vir.* 66:857 (1992), Raju and Huang, *J. Vir.* 65:2501 (1991), and Xiong *et al.*, *Science* 243:1188 (1989)), herpes viral vectors (*e.g.*, U.S. Patent Nos. 4,769,331, 4,859,587, 5,288,641 and 5,328,688), parvovirus vectors (Koering *et al.*, *Hum. Gene Therap.* 5:457 (1994)), pox virus vectors (Ozaki *et al.*, *Biochem. Biophys. Res. Comm.* 193:653 (1993), Panicali and Paoletti, *Proc. Nat'l Acad. Sci. USA* 79:4927 (1982)), pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:317 (1989), and Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86 (1989)), and retroviruses (*e.g.*, Baba *et al.*, *J. Neurosurg* 79:729 (1993), Ram *et al.*, *Cancer Res.* 53:83 (1993), Takamiya *et al.*, *J. Neurosci. Res* 33:493 (1992), Vile and Hart, *Cancer Res.* 53:962 (1993), Vile and Hart, *Cancer Res.* 53:3860 (1993), and Anderson *et al.*, U.S. Patent No. 5,399,346). Within various embodiments, either the viral vector itself, or a viral particle, which contains the viral vector may be utilized in the methods and compositions described below.

As an illustration of one system, adenovirus, a double-stranded DNA virus, is a well-characterized gene transfer vector for delivery of a heterologous nucleic acid molecule (for a review, see Becker *et al.*, *Meth. Cell Biol.* 43:161 (1994); Douglas and Curiel, *Science & Medicine* 4:44 (1997)). The adenovirus system offers several advantages including: (i) the ability to accommodate relatively large DNA inserts, (ii) the ability to be grown to high-titer, (iii) the ability to infect a broad range of mammalian cell types, and (iv) the ability to be used with many different promoters including

ubiquitous, tissue specific, and regulatable promoters. In addition, adenoviruses can be administered by intravenous injection, because the viruses are stable in the bloodstream.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene is deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell. When intravenously administered to intact animals, adenovirus primarily targets the liver. Although an adenoviral delivery system with an E1 gene deletion cannot replicate in the host cells, the host's tissue will express and process an encoded heterologous protein. Host cells will also secrete the heterologous protein if the corresponding gene includes a secretory signal sequence. Secreted proteins will enter the circulation from tissue that expresses the heterologous gene (e.g., the highly vascularized liver).

Moreover, adenoviral vectors containing various deletions of viral genes can be used to reduce or eliminate immune responses to the vector. Such adenoviruses are E1-deleted, and in addition, contain deletions of E2A or E4 (Lusky *et al.*, *J. Virol.* 72:2022 (1998); Raper *et al.*, *Human Gene Therapy* 9:671 (1998)). The deletion of E2b has also been reported to reduce immune responses (Amalfitano *et al.*, *J. Virol.* 72:926 (1998)). By deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. Generation of so called "gutless" adenoviruses, where all viral genes are deleted, are particularly advantageous for insertion of large inserts of heterologous DNA (for a review, see Yeh. and Perricaudet, *FASEB J.* 11:615 (1997)).

High titer stocks of recombinant viruses capable of expressing a therapeutic gene can be obtained from infected mammalian cells using standard methods. For example, recombinant herpes simplex virus can be prepared in Vero cells, as described by Brandt *et al.*, *J. Gen. Virol.* 72:2043 (1991), Herold *et al.*, *J. Gen. Virol.* 75:1211 (1994), Visalli and Brandt, *Virology* 185:419 (1991), Grau *et al.*, *Invest. Ophthalmol. Vis. Sci.* 30:2474 (1989), Brandt *et al.*, *J. Virol. Meth.* 36:209 (1992), and by Brown and MacLean (eds.), *HSV Virus Protocols* (Humana Press 1997).

Alternatively, an expression vector comprising a *TACI-immunoglobulin* gene can be introduced into a subject's cells by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987); Mackey *et al.*, *Proc. Nat'l Acad. Sci. USA* 85:8027 (1988)). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney,

and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (*e.g.*, hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration. For example, Aihara and Miyazaki, *Nature Biotechnology* 16:867 (1998), have demonstrated the use of *in vivo* electroporation for gene transfer into muscle.

In general, the dosage of a composition comprising a therapeutic vector having a *TACI-immunoglobulin* nucleotide acid sequence, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor. As an illustration, Horton *et al.*, *Proc. Nat'l Acad. Sci. USA* 96:1553 (1999), demonstrated that intramuscular injection of plasmid DNA encoding interferon- α produces potent antitumor effects on primary and metastatic tumors in a murine model.

A composition comprising viral vectors, non-viral vectors, or a combination of viral and non-viral vectors of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby vectors or viruses are combined in a mixture with a pharmaceutically acceptable carrier. As noted above, a composition, such as phosphate-buffered saline is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient subject. Other suitable carriers are well-known to those in the art (see, for example, *Remington's Pharmaceutical Sciences*, 19th Ed. (Mack Publishing Co. 1995), and *Gilman's the Pharmacological Basis of Therapeutics*, 7th Ed. (MacMillan Publishing Co. 1985)).

For purposes of therapy, a therapeutic gene expression vector, or a recombinant virus comprising such a vector, and a pharmaceutically acceptable carrier are administered to a subject in a therapeutically effective amount. A combination of an expression vector (or virus) and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient subject. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor.

When the subject treated with a therapeutic gene expression vector or a recombinant virus is a human, then the therapy is preferably somatic cell gene therapy. That is, the preferred treatment of a human with a therapeutic gene expression vector or a recombinant virus does not entail introducing into cells a nucleic acid molecule that can form part of a human germ line and be passed onto successive generations (*i.e.*, human germ line gene therapy).

10. **Production of Transgenic Mice**

Transgenic mice can be engineered to over-express nucleic acid sequences encoding TACI-immunoglobulin fusion proteins in all tissues, or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers of TACI-immunoglobulin fusion proteins can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess TACI receptor protein. Transgenic mice that over-express TACI-immunoglobulin fusion proteins also provide model bioreactors for production of TACI-immunoglobulin fusion proteins in the milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), *Strategies in Transgenic Animal Science* (ASM Press 1995), and Abbud and Nilson, "Recombinant Protein Expression in Transgenic Mice," in *Gene Expression Systems: Using Nature for the Art of Expression*, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

For example, a method for producing a transgenic mouse that expresses a nucleic acid sequence that encodes a TACI-immunoglobulin fusion protein can begin with adult, fertile males (studs) (B6C3f1, 2 to 8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2 to 8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4 to 5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2 to 4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8 IU/mouse of Pregnant Mare's Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) I.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) I.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection. Copulation is confirmed by the presence of a vaginal plug the morning following mating.

Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten's W640 medium (described, for example, by Menino and O'Claray, *Biol. Reprod.* 77:159 (1986), and
5 Dienhart and Downs, *Zygote* 4:129 (1996)) that has been incubated with 5% CO₂, 5% O₂, and 90% N₂ at 37°C. The eggs are then stored in a 37°C/5% CO₂ incubator until microinjection.

Ten to twenty micrograms of plasmid DNA containing a TACI-immunoglobulin fusion protein encoding sequence is linearized, gel-purified, and
10 resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the TACI-immunoglobulin fusion protein encoding sequences can encode a TACI polypeptide with deletion of amino acid residues 1 to 29 and 111 to 154 of SEQ ID NO:2, and an Fc5 immunoglobulin moiety.

15 Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO₂-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

20 Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO₂ incubator.

25 The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the
30 ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

35 With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day's injection are transferred into the recipient. The swollen ampulla is located and holding the oviduct

between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed
5 into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings
10 are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

Genomic DNA is prepared from the tail snips using, for example, a QIAGEN DNEASY kit following the manufacturer's instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a nucleic acid sequence encoding a
15 TACI-immunoglobulin fusion protein or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

To check for expression of a transgene in a live animal, a partial
20 hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the xiphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. An atraumatic clamp is used
25 to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with
30 suture and wound clips, and the animal's cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of TACI-immunoglobulin fusion protein mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

35 Using the general approach described above, transgenic mice have been produced that express significant levels of TACI-immunoglobulin fusion protein in milk. In this particular case, the TACI-immunoglobulin fusion protein encoding sequence

encoded a TACI polypeptide with deletion of amino acid residues 1 to 29 and 111 to 154 of SEQ ID NO:2, and an Fc5 immunoglobulin moiety.

5 The present invention, thus generally described, will be understood more readily by reference to the following examples, which are provided by way of illustration and is not intended to be limiting of the present invention.

EXAMPLE 1

Construction of Nucleic Acid Molecules That Encode TACI-Fc Proteins

10 Nucleic acid molecules encoding human TACI were obtained during the expression cloning of the receptors for ZTNF4 as described by Gross *et al.*, *Nature* 404:995 (2000). The coding sequences contained in the TACI-Fc expression constructs were generated by overlap PCR, using standard techniques (see, for example, Horton *et al.*, *Gene* 77:61 (1989)). Human TACI cDNA and Fc cDNA were used as starting
15 templates for the PCR amplifications. PCR primers were designed to yield the desired coding sequence 5' and 3' ends and to introduce restriction enzyme recognition sites to facilitate insertion of these coding sequences into the expression vectors. The TACI-Fc coding sequences were inserted into expression vectors that included a functional murine *dihydrofolate reductase* gene. One expression vector also contained a cytomegalovirus
20 promoter to direct the expression of the recombinant protein transgene, an immunoglobulin intron, a tissue plasminogen activator signal sequence, an internal ribosome entry sequence, a deleted CD8 cistron for surface selection of transfected cells, and yeast expression elements for growth of the plasmid in yeast cells.

One approach that was used to produce TACI-Fc fusion proteins is
25 illustrated by the method used to construct TACI-Fc4. Other TACI-Fc fusion proteins were produced by inserting nucleotide sequences that encode a TACI-Fc fusion protein into a mammalian expression vector, and introducing that expression vector into mammalian cells.

30 **A. Ig γ 1 Fc4 Fragment Construction**

To prepare the TACI-Fc4 fusion protein, the Fc region of human IgG1 (the hinge region and the CH₂ and CH₃ domains) was modified to remove Fc γ 1 receptor (Fc γ RI) and complement (C1q) binding functions. This modified version of human IgG1 Fc was designated "Fc4."

The Fc region was isolated from a human fetal liver library (Clontech) PCR using oligo primers 5' ATCAGCGGAA TTCAGATCTT CAGACAAAAC TCACACATGC CCAC 3' (SEQ ID NO:7) and 5' GGCAGTCTCT AGATCATTTA CCCGGAGACA GGGAG 3' (SEQ ID NO:8). Mutations within the Fc region were introduced by PCR to reduce FcγRI binding. The FcγRI binding site (Leu-Leu-Gly-Gly; amino acid residues 38 to 41 of SEQ ID NO:6, which correspond to EU index positions 234 to 237) was mutated to Ala-Glu-Gly-Ala to reduce FcγR1 binding (see, for example, Duncan *et al.*, *Nature* 332:563 (1988); Baum *et al.*, *EMBO J.* 13:3992 (1994)). Oligonucleotide primers 5' CCGTGCCAG CACCTGAAGC CGAGGGGGCA CCGTCAGTCT TCCTCTTCCC C 3' (SEQ ID NO:9) and 5' GGATTCTAGA TTATTACCC GGAGACAGGG A 3' (SEQ ID NO:10) were used to introduce the mutation. To a 50 μl final volume was added 570 ng of IgFc template, 5 μl of 10x Pfu reaction Buffer (Stratagene), 8 μl of 1.25 mM dNTPs, 31 μl of distilled water, 2 μl of 20 mM oligonucleotide primers. An equal volume of mineral oil was added and the reaction was heated to 94°C for 1 minute. Pfu polymerase (2.5 units, Stratagene) was added followed by 25 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute followed by a 7 minute extension at 72°C. The reaction products were fractionated by electrophoresis, and the band corresponding to the predicted size of about 676 base pairs was detected. This band was excised from the gel and recovered using a QIAGEN QIAquick™ Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

PCR was also used to introduce a mutation of Ala to Ser (amino acid residue 134 of SEQ ID NO:6, which corresponds to EU index position 330) and Pro to Ser (amino acid residue 135 of SEQ ID NO:6, which corresponds to EU index position 331) to reduce complement C1q binding or complement fixation (Duncan and Winter, *Nature* 332:788 (1988)). Two first round reactions were performed using the FcγRI binding site-mutated IgFc sequence as a template. To a 50 μl final volume was added 1 μl of FcγRI binding site mutated IgFc template, 5 μl of 10x Pfu Reaction Buffer (Stratagene), 8 μl of 1.25 mM dNTPs, 31 of μl distilled water, 2 μl of 20 mM 5' GGTGGCGGCT CCCAGATGGG TCCTGTCCGA GCCCAGATCT TCAGACAAAA CTCAC 3' (SEQ ID NO:11), a 5' primer beginning at nucleotide 36 of SEQ ID NO:5, and 2 μl of 20 mM 5' TGGGAGGGCT TTGTTGGA 3' (SEQ ID NO:12), a 3' primer beginning at the complement of nucleotide 405 of SEQ ID NO:5. The second reaction contained 2 μl each of 20 mM stocks of oligonucleotide primers 5' TCCAACAAAG CCCTCCCATC CTCCATCGAG AAAACCATCT CC 3' (SEQ ID NO:13), a 5' primer beginning at nucleotide 388 of SEQ ID NO:5 and 5' GGATGGATCC ATGAAGCACC TGTGGTTCTT CCTCCTGCTG GTGGCGGCTC CCAGATG 3' (SEQ ID NO:14), a 3' primer, to introduce the Ala to Ser mutation, *Xba*I restriction site and stop codon. An

equal volume of mineral oil was added and the reactions were heated to 94°C for 1 minute. Pfu polymerase (2.5 units, Stratagene) was added followed by 25 cycles at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 2 minutes followed by a 7 minute extension at 72°C. The reaction products were fractionated by electrophoresis, and bands corresponding to the predicted sizes, about 370 and about 395 base pairs respectively, were detected. The bands were excised from the gel and extracted using a QIAGEN QIAquick™ Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

A second round reaction was performed to join the above fragments and add the 5' *Bam*HI restriction site and a signal sequence from the human immunoglobulin JBL 2'C_L heavy chain variable region (Cogne *et al.*, *Eur. J. Immunol.* 18:1485 (1988)). To a 50 µl final volume was added 30 µl of distilled water, 8 µl of 1.25 mM dNTPs, 5 µl of 10x Pfu polymerase reaction buffer (Stratagene) and 1 µl each of the two first two PCR products. An equal volume of mineral oil was added and the reaction was heated to 94°C for 1 minute. Pfu polymerase (2.5 units, Stratagene) was added followed by 5 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 2 minutes. The temperature was again brought to 94°C and 2 µl each of 20 mM stocks of 5' GGATGGATCC ATGAAGCACC TGTGGTTCTT CCTCCTGCTG GTGGCGGCTC CCAGATG 3' (SEQ ID NO:14), a 5' primer beginning at nucleotide 1 of SEQ ID NO:5, and 5' GGATTCTAGA TTATTTACCC GGAGACAGGG A 3' (SEQ ID NO:10) were added followed by 25 cycles at 94°C for 30 seconds, 55°C for 30 seconds and 72°C for 2 minutes, and a final 7 minute extension at 72°C. A portion of the reaction was visualized using gel electrophoresis. A 789 base pair band corresponding the predicted size was detected.

B. TACI-Fc4 Expression Vector Construction

Expression plasmids comprising a coding region for TACI-Fc4 fusion protein were constructed via homologous recombination in yeast. A fragment of TACI cDNA was isolated using PCR that included the polynucleotide sequence from nucleotide 14 to nucleotide 475 of SEQ ID NO:1. The two primers used in the production of the TACI fragment were: (1) a primer containing 40 base pairs of the 5' vector flanking sequence and 17 base pairs corresponding to the amino terminus of the TACI fragment (5' CTCAGCCAGG AAATCCATGC CGAGTTGAGA CGCTTCCGTA GAATGAGTGG CCTGGGCCG 3'; SEQ ID NO:15); (2) 40 base pairs of the 3' end corresponding to the flanking Fc4 sequence and 17 base pairs corresponding to the carboxyl terminus of the TACI fragment (5' GCATGTGTGA GTTTTGTCTG

AAGATCTGGG CTCCTTCAGC CCCGGGAG 3'; SEQ ID NO:16). To a 100 µl final volume was added 10 ng of TACI template, 10 µl of 10x Taq polymerase Reaction Buffer (Perkin Elmer), 8 µl of 2.5 mM dNTPs, 78 µl of distilled water, 2 µl each of 20 mM stocks of the oligonucleotide primers, and Taq polymerase (2.5 units, Life Technology). An equal volume of mineral oil was added and the reaction was heated to 94°C for 2 minutes, followed by 25 cycles at 94°C for 30 seconds, 65°C for 30 seconds, 65°C for 30 seconds, 72°C for 1 minute followed by a 5 minute extension at 72°C.

The fragment containing the cDNA encoding the Fc4 fragment was constructed in a similar manner. The two primers used in the production of the Fc4 fragment were (upstream and downstream), an oligonucleotide primer containing 40 base pairs of the 5' TACI flanking sequence and 17 base pairs corresponding to the amino terminus of the Fc4 fragment (5' GCACAGAGGC TCAGAAGCAA GTCCAGCTCT CCCGGGGCTG AAGGAGCCCA GATCTTCAGA 3'; SEQ ID NO:17); and an oligonucleotide primer containing 40 base pairs of the 3' end corresponding to the flanking vector sequence and 17 base pairs corresponding to the carboxyl terminus of the Fc4 fragment (5' GGGGTGGGTA CAACCCCA GCTGTTTAA TCTAGATTAT TTACCCGGAG ACAGGG 3'; SEQ ID NO:18). To a 100 µl final volume was added 10 ng of Fc4 template described above, 10 µl 10x Taq polymerase Reaction Buffer (Perkin Elmer), 8 µl of 2.5 mM dNTPs, 78 µl of distilled water, 2 µl each of 20 mM stocks of the oligonucleotides, and Taq polymerase (2.5 units, Life Technology). An equal volume of mineral oil was added and the reaction was heated to 94°C for 2 minutes, then 25 cycles at 94°C for 30 seconds, 65°C for 30 seconds, 72°C for 1 minute followed by a 5 minute extension at 72°C.

Ten microliters of each of the 100 µl PCR reactions described above were run on a 0.8% LMP agarose gel (Seaplaque GTG) with 1x TBE buffer for analysis. The remaining 90 µl of each PCR reaction was precipitated with the addition of 5 µl of 1 M sodium chloride and 250 µl of absolute ethanol. The plasmid pZMP6 was cleaved with *Sma*I to linearize it at the polylinker. Plasmid pZMP6 was derived from the plasmid pCZR199 (American Type Culture Collection, Manassas, VA, ATCC# 98668) and is a mammalian expression vector containing an expression cassette having the cytomegalovirus immediate early promoter, a consensus intron from the variable region of mouse immunoglobulin heavy chain locus, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a *dihydrofolate reductase* gene and the SV40 terminator. The vector pZMP6 was constructed from pCZR199 by

replacement of the metallothionein promoter with the cytomegalovirus immediate early promoter, and the Kozac sequences at the 5' end of the open reading frame.

One hundred microliters of competent yeast cells (*S. cerevisiae*) were combined with 10 μ l containing approximately 1 μ g of the TACI extracellular domain and the Fc4 PCR fragments, and 100 ng of *Sma*I digested pZMP6 vector and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixtures were electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μ F. To each cuvette was added 600 μ l of 1.2 M sorbitol and the yeast were plated in two 300 μ l aliquots onto to URA-D plates and incubated at 30°C.

After about 48 hours, the Ura⁺ yeast transformants from a single plate were resuspended in 1 ml of water and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 1 ml of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). Five hundred microliters of the lysis mixture was added to an Eppendorf tube containing 300 μ l acid washed glass beads and 200 μ l phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA precipitated with 600 μ l of ethanol, followed by centrifugation for 10 minutes at 4°C. The DNA pellet was resuspended in 100 μ l of water.

Transformation of electrocompetent *E. coli* cells (DH10B, GibcoBRL) was performed with 0.5-2 ml yeast DNA prep and 40 μ l of DH10B cells. The cells were electropulsed at 2.0 kV, 25 mF and 400 ohms. Following electroporation, 1 ml of SOC (2% Bacto-Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) were plated in 250 μ l aliquots on four LB AMP plates (LB broth (Lennox), 1.8% Bacto-Agar (Difco), 100 mg/L Ampicillin).

Individual clones harboring the correct expression construct for TACI-Fc4 were identified by restriction digest to verify the presence of the insert and to confirm that the various DNA sequences have been joined correctly to one another. The insert of positive clones were subjected to sequence analysis. Larger scale plasmid DNA is isolated using the Qiagen Maxi kit (Qiagen) according to manufacturer's instructions.

C. Construction of Fc5, Fc6, and Fc7

In Fc5, the Arg residue at EU index position 218 was changed back to a Lys residue. Wild-type human Ig γ 1 contains a lysine at this position. Briefly, nucleic acid molecules encoding Fc5 were produced using oligonucleotide primers 5'

GAGCCCAAATCTTCAGACAAAACCTCACACATGCCCA 3' (SEQ ID NO:19) and 5' TAATTGGCGCGCCTCTAGATTATTTACCCGGAGACA 3' (SEQ ID NO:20). The conditions of the PCR amplification were as follows. To a 50 µl final volume was added 236 ng of Fc4 template, 5 µl of 10 Pfu reaction Buffer (Stratagene), 4 µl of 2.5 mM dNTPs, 1 µl of 20 µM of each of the oligonucleotides, and 1 µl of Pfu polymerase (2.5 units, Stratagene). The amplification thermal profile consisted of 94°C for 2 minutes, 5 cycles at 94°C for 15 seconds, 42°C for 20 seconds, 72°C for 45 seconds, 20 cycles at 94°C for 15 seconds, 72°C for 1 minute 20 seconds, followed by a 7 minute extension at 72°C. The reaction product was fractionated by agarose gel electrophoresis, and the band corresponding to the predicted size of about 718 base pairs was detected. The band was excised from the gel and recovered using a QIAGEN QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions.

Fc6 is identical to Fc5 except that the carboxyl terminal lysine codon has been eliminated. As in Fc4 and Fc5 above, the stop codon in the Fc6 sequence was changed to TAA. Fc6 was generated from template DNA that encoded Fc5 using oligonucleotide primers 5' GAGCCCAAAT CTTCAGACAA AACTCACACA TGCCCA 3' (SEQ ID NO:19) and 5' GGCGCGCCTC TAGATTAACC CGGAGACAGG GAGAGGC 3' (SEQ ID NO:21).

Fc7 is identical to the wild-type γ1 Fc except for an amino acid substitution at EU index position Asn 297 located in the C_H2 domain. Asn 297 was mutated to a Gln residue to prevent the attachment of N-linked carbohydrate at that residue position. As above, the stop codon in the Fc7 sequence was changed to TAA. Fc7 was generated by overlap PCR using a wild-type human IgGγ1 Fc cDNA as the template and oligonucleotide primers 5' GAGCCCAAATCTTGCGACAAAACCTCACA 3' (SEQ ID NO:22) and 5' GTACGTGCTTTGGTACTGCTCCTCCCGCGGCTT 3' (SEQ ID NO:23) to generate the 5' half of Fc7, and oligonucleotide primers 5' CAGTACCAAAGCACGTACCGTGTGGTCA 3' (SEQ ID NO:24) and 5' TAATTGGCGCGCCTCTAGATTATTTACCCGGAGACA 3' (SEQ ID NO:20) to generate the 3' half of Fc7. The two PCR products were combined and amplified using oligonucleotide primers 5' GAGCCCAAATCTTGCGACAAAACCTCACA 3' (SEQ ID NO:22) and 5' TAATTGGCGCGCCTCTAGATTATTTACCCGGAGACA 3' (SEQ ID NO:20).

All the resultant PCR products were gel purified, cloned, and verified by DNA sequence analysis.

D. Construction of Amino-Truncated TACI-Fc Fusion Proteins

Four amino terminal truncated versions of TACI-Fc were generated. All four had a modified human tissue plasminogen activator signal sequence (SEQ ID NO:25) fused to amino acid residue number 30 of SEQ ID NO:2. However, the four proteins differed in the location of point in which the Fc5 was fused to the TACI amino acid sequence of SEQ ID NO:2. Table 3 outlines the structures of the four fusion proteins.

Table 3
TACI Fusion Proteins

Designation of TACI-Fc	TACI amino acid residues
TACI(d1-29)-Fc5	30 to 154 of SEQ ID NO:2
TACI(d1-29, d107-154)-Fc5	30 to 106 of SEQ ID NO:2
TACI(d1-29, d111-154)-Fc5	30 to 110 of SEQ ID NO:2
TACI(d1-29, d120-154)-Fc5	30 to 119 of SEQ ID NO:2

Protein encoding expression cassettes were generated by overlap PCR using standard techniques (see, for example, Horton *et al.*, *Gene* 77:61 (1989)). A nucleic acid molecule encoding TACI and a nucleic acid molecule encoding Fc5 were used as PCR templates. Oligonucleotide primers are identified in Tables 4 and 5.

Table 4
Oligonucleotide Primers Used to Produce TACI Fusion Proteins

Designation of TACI-Fc	Oligonucleotide Designations			
	5' TACI	3' TACI	5' Fc5	3' Fc5
TACI(d1-29)-Fc5	ZC24,903	ZC24,955	ZC24,952	ZC24,946
TACI(d1-29, d107-154)-Fc5	ZC24,903	ZC24,951	ZC24,949	ZC24,946
TACI(d1-29, d111-154)-Fc5	ZC24,903	ZC28,978	ZC28,979	ZC24,946
TACI(d1-29, d120-154)-Fc5	ZC24,903	ZC28,981	ZC28,980	ZC24,946

Table 5
Oligonucleotide Sequences

Primer	Nucleotide Sequence	SEQ ID NO.
ZC24,903	5' TATTAGGCCGGCCACCATGGATGCAATGA 3'	40
ZC24,955	5' TGAAGATTTGGGCTCCTTGAGACCTGGGA 3'	41
ZC24,952	5' TCCCAGGTCTCAAGGAGCCCAAATCTTCA 3'	42
ZC24,946	5' TAATTGGCGCGCCTCTAGATTATTTACCCGGAGACA 3'	20
ZC24,951	5' TGAAGATTTGGGCTCGTTCTCACAGAAGTA 3'	43
ZC24,949	5' ATACTTCTGTGAGAACGAGCCCAAATCTTCA 3'	44
ZC28,978	5' TTTGGGCTCGCTCCTGAGCTTGTTCTCACA 3'	45
ZC28,979	5' CTCAGGAGCGAGCCCAAATCTTCAGACA 3'	46
ZC28,981	5' TTTGGGCTCCCTGAGCTCTGGTGGAA 3'	47
ZC28,980	5' GAGCTCAGGGAGCCCAAATCTTCAGACA 3'	48

5 The first round of PCR amplifications consisted of two reactions for each of the four amino terminal truncated versions. The two reactions were performed separately using the 5' and 3' TACI oligonucleotides in one reaction, and the 5' and 3' Fc5 oligonucleotides in another reaction for each version. The conditions of the first round PCR amplification were as follows. To a 25 μ l final volume was added approximately 100 ng template DNA, 2.5 μ l 10x Pfu reaction Buffer (Stratagene), 2 μ l of 2.5 mM dNTPs, 0.5 μ l of 20 μ M each 5' oligonucleotide and 3' oligonucleotide, and 0.5 μ l Pfu polymerase (2.5 units, Stratagene). The amplification thermal profile consisted of 94°C for 3 minutes, 35 cycles at 94°C for 15 seconds, 50°C for 15 seconds, 72°C for 2 minutes, followed by a 2 minute extension at 72°C. The reaction products were fractionated by agarose gel electrophoresis, and the bands corresponding to the predicted sizes were excised from the gel and recovered using a QIAGEN QIAQUICK Gel Extraction Kit (Qiagen), according to the manufacturer's instructions.

20 The second round of PCR amplification, or overlap PCR amplification reaction, was performed using the gel purified fragments from the first round PCR as DNA template. The conditions of the second round PCR amplification were as follows. To a 25 μ l final volume was added approximately 10 ng template DNA each of the TACI fragment and the Fc5 fragment, 2.5 μ l 10x Pfu reaction Buffer (Stratagene), 2 μ l of 2.5 mM dNTPs, 0.5 μ l of 20 μ M each ZC24,903 (SEQ ID NO:40) and ZC24,946 (SEQ ID NO:20) and 0.5 μ l Pfu polymerase (2.5 units, Stratagene). The amplification thermal

profile consisted of 94°C for 1 minute, 35 cycles at 94°C for 15 seconds, 55°C for 15 seconds, 72°C for 2 minutes, followed by a 2 minute extension at 72°C. The reaction products were fractionated by agarose gel electrophoresis, and the bands corresponding to the predicted sizes were excised from the gel and recovered using a QIAGEN
5 QIAQUICK Gel Extraction Kit (Qiagen), according to the manufacturer's instructions.

Each of the four versions of the amino terminal truncated TACI-Fc PCR products were separately cloned using Invitrogen's ZEROBLUNT TOPO PCR Cloning Kit following the manufacturer's recommended protocol. Table 6 identifies the nucleotide and amino acid sequences of these TACI-Fc constructs.

10

Table 6
Sequences of TACI-Fc Variants

Designation of TACI-Fc	SEQ ID Nos.	
	Nucleotide	Amino Acid
TACI(d1-29)-Fc5	49	50
TACI(d1-29, d107-154)-Fc5	51	52
TACI(d1-29, d111-154)-Fc5	53	54
TACI(d1-29, d120-154)-Fc5	55	56

15

After the nucleotide sequences were verified, plasmids comprising each of the four versions of the amino terminal truncated TACI-Fc fusions were digested with *FseI* and *AscI* to release the amino acid encoding segments. The *FseI* - *AscI* fragments were ligated into a mammalian expression vector containing a CMV promoter and an SV40 poly A segment. Expression vectors were introduced into Chinese hamster ovary
20 cells as described below.

EXAMPLE 2

Production of TACI-Fc Proteins by Chinese Hamster Ovary Cells

The TACI-Fc expression constructs were used to transfect, via electroporation, suspension-adapted Chinese hamster ovary (CHO) DG44 cells grown in animal protein-free medium (Urlaub *et al.*, *Som. Cell. Molec. Genet.* 12:555 (1986)). CHO DG44 cells lack a functional *dihydrofolate reductase* gene due to deletions at both *dihydrofolate reductase* chromosomal locations. Growth of the cells in the presence of increased concentrations of methotrexate results in the amplification of the *dihydrofolate reductase* gene, and the linked recombinant protein-encoded gene on the expression construct.

CHO DG44 cells were passaged in PFCHO media (JRH Biosciences, Lenexa, KS), 4 mM L-Glutamine (JRH Biosciences), and 1x hypoxanthine-thymidine supplement (Life Technologies), and the cells were incubated at 37°C and 5% CO₂ in Corning shake flasks at 120 RPM on a rotating shaker platform. The cells were transfected separately with linearized expression plasmids. To ensure sterility, a single ethanol precipitation step was performed on ice for 25 minutes by combining 200 µg of plasmid DNA in an Eppendorf tube with 20 µl of sheared salmon sperm carrier DNA (5' → 3' Inc. Boulder, CO, 10 mg/ml), 22 µl of 3M NaOAc (pH 5.2), and 484 µl of 100% ethanol (Gold Shield Chemical Co., Hayward, CA). After incubation, the tube was centrifuged at 14,000 RPM in a microfuge placed in a 4°C cold room, the supernatant removed and the pellet washed twice with 0.5 ml of 70% ethanol and allowed to air dry.

The CHO DG44 cells were prepared while the DNA pellet was drying by centrifuging 10⁶ total cells (16.5 ml) in a 25 ml conical centrifuge tube at 900 RPM for 5 minutes. The CHO DG44 cells were resuspended into a total volume of 300 µl of PFCHO growth media, and placed in a Gene-Pulser Cuvette with a 0.4 cm electrode gap (Bio-Rad). The DNA, after approximately 50 minutes of drying time, was resuspended into 500 µl of PFCHO growth media and added to the cells in the cuvette so that the total volume did not exceed 800 µl and was allowed to sit at room temperature for 5 minutes to decrease bubble formation. The cuvette was placed in a BioRad Gene Pulser II unit set at 0.296 kV (kilovolts) and 0.950 HC (high capacitance) and electroporated immediately.

The cells were incubated 5 minutes at room temperature before placement in 20 ml total volume of PFCHO media in a CoStar T-75 flask. The flask was placed at 37°C and 5% CO₂ for 48 hours when the cells were then counted by hemocytometer utilizing trypan blue exclusion and put into PFCHO selection media without hypoxanthine-thymidine supplement and containing 200 mM methotrexate (Cal Biochem).

Upon recovery of the methotrexate selection process, the conditioned media containing the secreted TACI-Fc proteins were examined by Western Blot analysis.

EXAMPLE 3

Structural Analysis of TACI-Fc Proteins

In certain cases, TACI-Fc fusion proteins were partially purified before analysis. Conditioned medium from Chinese hamster ovary cultures was sterile-filtered through a 0.22 μ m filter and the TACI-Fc protein was captured on a protein A column. The protein A-bound material was eluted and passed over an S-200 size exclusion column for final purification.

Western blot analysis was performed on both conditioned cell medium and purified protein to assess the structural stability of the TACI-Fc proteins. Briefly, protein or supernatant samples were transferred to nitrocellulose membranes and the TACI-Fc proteins were detected using peroxidase conjugated goat anti-mouse IgG2a (Boehringer Mannheim), or peroxidase conjugated goat anti-human IgG Fc specific antisera (Pierce).

Amino terminal amino acid sequence analyses were performed on Models 476A and 494 Protein Sequencer Systems from Perkin Elmer Applied Biosystems Division (Foster City, CA). Data analysis was performed with Applied Biosystems Model 610A Data Analysis System for Protein Sequencing, version 2.1a (Applied Biosystems, Inc.). Most supplies and reagents used were from Applied Biosystems, Inc.

EXAMPLE 4

Functional Analysis of TACI-Fc Proteins

Two approaches were used to examine the binding characteristics of four TACI-Fc proteins with ZTNF4. One approach measured the ability of the TACI-Fc constructs to compete with TACI-coated plates for binding of ^{125}I -labeled ZTNF4. In the second approach, increasing concentrations of ^{125}I labeled ZTNF4 were incubated with each of the TACI-Fc constructs, and the radioactivity associated with precipitated ZTNF4-TACI-Fc complexes was determined. The TACI-Fc fusion proteins had TACI moieties that lacked the first 29 amino acid residues of the amino acid sequence of SEQ ID NO:2. One of the fusion proteins had a TACI moiety with an intact stalk region (TACI (d1-29)-Fc5), whereas three of the TACI-Fc fusion proteins had TACI moieties with various deletions in the stalk region (TACI (d1-29, d107-154)-Fc5; TACI (d1-29, d111-154)-Fc5; TACI (d1-29, d120-154)-Fc5).

A. Competitive Binding Assay

ZTNF4 was radiolabeled with Iodobeads (Pierce), following standard methods. Briefly, 50 μg of the ZTNF4 was iodinated with 4 mCi of ^{125}I using a single Iodobead. The reaction was quenched with a 0.25% solution of bovine serum albumin, and the free ^{125}I was removed by gel filtration using a PD-10 column (Pierce). The specific radioactivity of ^{125}I -ZTNF4 preparations was determined by trichloroacetic acid precipitation before and after the desalting step.

An N-terminal fragment of the TACI receptor, designated as "TACI-N," was added to 96-well plates (100 μl at 0.1 $\mu\text{g}/\text{ml}$), and incubated overnight at 4°C. The plates were washed, blocked with Superblock (Pierce), and washed again. The TACI-Fc constructs, at various concentrations ranging from 0 to 11.5 ng/ml, were mixed with a fixed concentration of ^{125}I -ZTNF4 (20 ng/ml), and incubated for 2 hours at 37°C on the plate coated with TACI-N. Controls contained either TACI-N in solution, or lacked TACI-Fc. After incubation, the plates were washed and counted. Each determination was performed in triplicate.

The results showed that all TACI-Fc constructs inhibited ^{125}I -ZTNF4 binding completely at concentrations of about 100 ng/ml or greater. The TACI-Fc proteins, TACI (d1-29)-Fc5, TACI (d1-29, d111-154)-Fc5, and TACI (d1-29, d120-154)-Fc5, were more effective inhibitors than the TACI-Fc construct, TACI (d1-29, d107-154)-Fc5. An Fc fragment alone did not inhibit binding. IC_{50} values were calculated for each construct in three experiments. The average values for the constructs were: TACI

(d1-29)-Fc5: 2.07 nM; TACI (d1-29, d107-154)-Fc5: 4.95 nM; TACI (d1-29, d111-154)-Fc5: 2.31 nM; and TACI (d1-29, d120-154)-Fc5: 2.21 nM.

B. Solution Binding Assay

At a concentration of 0.05 nM, each TACI-Fc construct was incubated with 0.4 pM to 1.5 nM 125 I-ZTNF4 for 30 minutes at room temperature in a total volume of 0.25 ml/tube. A Pansorbin (Staph A) suspension was added to each tube, and after 15 minutes, the samples were centrifuged, washed twice, and the pellets counted. Nonspecific binding was determined by the addition of 130 nM unlabeled ZTNF4 to the 125 I-ZTNF4/TACI-Fc mix. Specific binding was calculated by subtracting the cpm bound in the presence of unlabeled ZTNF4 from the total cpm bound at each concentration of 125 I-ZTNF4. Each determination was performed in triplicate. Binding constants were calculated using GraphPad Prism software (MacIntosh v. 3.0).

Figure 4 illustrates the specific binding of 125 I-ZTNF4 to the various TACI-Fc constructs. Binding appeared to approach saturation with each construct, and was significantly higher for constructs TACI (d1-29)-Fc5, TACI (d1-29, d111-154)-Fc5, TACI (d1-29, d120-154)-Fc5, as compared with the binding of TACI (d1-29, d107-154)-Fc5. Bmax and Kd values were calculated, and the results are summarized in Table 7.

Table 7
Binding of 125 I-ZTNF4 to TACI-Fc Constructs

TACI-Fc Construct	Kd (nM)	Bmax (nM)
TACI (d1-29)-Fc5	0.134	0.023
TACI (d1-29, d107-154)-Fc5	0.121	0.010
TACI (d1-29, d111-154)-Fc5	0.115	0.018
TACI (d1-29, d120-154)-Fc5	0.092	0.021

EXAMPLE 5

Measurement of Circulating ZTNF4

Levels of ZTNF4 in individuals with a disease condition (such as SLE, rheumatoid arthritis for example) relative to normal individuals were determined using an electrochemiluminescence assay. A standard curve prepared from soluble, human

ZTNF4 at 10 ng/ml, 1 ng/ml, 0.1 ng/ml, 0.01 ng/ml and 0 ng/ml was prepared in ORIGIN buffer (Igen, Gaithersburg, MD). Serum samples were diluted in ORIGIN buffer. The standards and samples were incubated at room temperature for two hours with biotinylated rabbit anti-human ZTNF4-NF BV antibody diluted to 1 µg/ml in Origin Assay Buffer (IGEN) and ruthenylated rabbit anti-human ZTNF4-NF BV polyclonal antibody diluted to 1 µg/ml in Origin Assay Buffer (IGEN). Following the incubation the samples were vortexed and 0.4 mg/ml streptavidin Dynabeads (Dyna, Oslo, Norway) were added to each of the standards and samples at 50 µl/tube and incubated for 30 minutes at room temperature. Samples were then vortexed and samples were read on an Origin Analyzer (Igen) according to manufacturer's instructions. The Origin assay is based on electrochemiluminescence and produces a readout in ECL. In one study, an elevated level of ZTNF4 was detected in the serum samples from both NZBWF1/J, and MRL/Mpj-Fas^{lpr} mice, which have progressed to advanced stages of glomerulonephritis and autoimmune disease.

The ORIGIN ASSAY was also used to measure levels of ZTNF4 in the blood of SLE patients, relative to circulating levels in normal individuals. A standard curve prepared from soluble, human ZTNF4 at 10 ng/ml, 1 ng/ml, 0.1 ng/ml, 0.01 ng/ml and 0 ng/ml was prepared in ORIGIN buffer (Igen). All patient samples were run in triplicate with a 25 µl final volume. The standards and samples were incubated at room temperature for two hours with a capture antibody, biotinylated rabbit anti-human ZTNF4-NF BV polyclonal antibody, diluted to 1 µg/ml in Origin Assay Buffer (IGEN) and a detection antibody, ruthenylated rabbit anti-human ZTNF4-NF BV polyclonal antibody, diluted to 1 µg/ml in Origin Assay Buffer (IGEN). Following the incubation the samples were vortexed, and 0.4 mg/ml streptavidin Dynabeads (Dyna) was added to each of the standards and samples at 50 µl/tube and incubated for 30 minutes at room temperature. Samples were then vortexed, and analyzed using an Origin 1.5 Analyzer (Igen) according to manufacturer's instructions.

This assay included 28 normal control samples and samples from 20 patients diagnosed with SLE. Elevated levels of ZTNF4 were observed in the serum of patients diagnosed with SLE, as compared with normal control serum donors. ZTNF4 levels were calculated as a fold increase of ZTNF4 levels in the patient or control samples as compared to an arbitrary human reference serum sample. The average of the 28 control samples was 1.36 fold over the human reference sample and the average of the 20 SLE patient samples was 4.92. Seven out of the 20 SLE patients had ZTNF4 levels that were two fold over the average of the control samples, whereas there was only one control individual that had a greater than two fold level over the control average.

CLAIMS

What is claimed is

1. The use of a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion protein for the manufacture a medicament for inhibiting the proliferation of tumor cells, wherein the TACI-immunoglobulin fusion protein comprises:

(a) a TACI receptor moiety that consists of a fragment of a polypeptide that has the amino acid sequence of amino acid residues 30 to 154 of SEQ ID NO:2, wherein the TACI receptor moiety comprises at least one of (i) amino acid residues 34 to 66 of SEQ ID NO:2, and (ii) amino acid residues 71 to 104 of SEQ ID NO:2, and wherein the TACI receptor moiety binds at least one of ZTNF2 or ZTNF4, and

(b) an immunoglobulin moiety comprising a constant region of an immunoglobulin.

2. The use of claim 1, wherein the TACI receptor moiety comprises amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2.

3. The use of claim 1, wherein the TACI receptor moiety comprises amino acid residues 34 to 104 of SEQ ID NO:2.

4. The use of claim 1, wherein the TACI receptor moiety has the amino acid sequence of amino acid residues 30 to 110 of SEQ ID NO:2

5. The use of claim 1, wherein the immunoglobulin moiety comprises a heavy chain constant region.

6. The use of claim 5, wherein the immunoglobulin moiety comprises a human heavy chain constant region.

7. The use of claim 6, wherein the heavy chain constant region is an IgG1 heavy chain constant region.

8. The use of claim 7, wherein the immunoglobulin moiety is an IgG1 Fc fragment that comprises C_{H2}, and C_{H3} domains.

9. The use of claim 8, wherein the immunoglobulin moiety is an IgG1 Fc fragment comprising the amino acid sequence of SEQ ID NO:33.

10. The use of claim 9, wherein TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

11. The use of claim 1, wherein the TACI-immunoglobulin fusion protein is a dimer.

12. The use of claim 1, wherein the composition is administered to cells cultured *in vitro*.

13. The use of claim 1, wherein the composition is a pharmaceutical composition that comprises the TACI-immunoglobulin fusion protein and a pharmaceutically acceptable carrier, and wherein the pharmaceutical composition is administered to a mammalian subject, which has a tumor.

14. The use of claim 13, wherein the administration of the pharmaceutical composition inhibits the proliferation of B lymphocytes in the mammalian subject.

15. The use of a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion protein for the manufacture a medicament for inhibiting the proliferation of tumor cells in a mammalian subject, wherein the TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

16. The use of claim 15, wherein the TACI-immunoglobulin fusion protein is a dimer.

17. A fusion protein, comprising:

(a) a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI) receptor moiety, wherein the TACI receptor moiety consists of a fragment of a polypeptide that has the amino acid sequence of amino acid residues 30 to 154 of SEQ ID NO:2, wherein the TACI receptor moiety comprises at least one of (i) amino acid residues 34 to 66 of SEQ ID NO:2, and (ii) amino acid

residues 71 to 104 of SEQ ID NO:2, and wherein the TACI receptor moiety binds at least one of ZTNF2 or ZTNF4, and

(b) an immunoglobulin moiety that comprises a constant region of an immunoglobulin.

18. The fusion protein of claim 17, wherein the TACI receptor moiety comprises amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2.

19. The fusion protein of claim 17, wherein the TACI receptor moiety comprises amino acid residues 34 to 104 of SEQ ID NO:2.

20. The fusion protein of claim 17, wherein the TACI receptor moiety has the amino acid sequence of amino acid residues 30 to 110 of SEQ ID NO:2

21. The fusion protein of claim 17, wherein the immunoglobulin moiety comprises a heavy chain constant region.

22. The fusion protein of claim 21, wherein the immunoglobulin moiety comprises a human heavy chain constant region.

23. The fusion protein of claim 22, wherein the heavy chain constant region is an IgG1 heavy chain constant region.

24. The fusion protein of claim 23, wherein the immunoglobulin moiety is an IgG1 Fc fragment that comprises C_{H2}, and C_{H3} domains.

25. The fusion protein of claim 24, wherein the immunoglobulin moiety is an IgG1 Fc fragment comprising the amino acid sequence of SEQ ID NO:33.

26. The fusion protein of claim 25, wherein TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

27. The fusion protein of claim 17, wherein the TACI-immunoglobulin fusion protein is a dimer.

28. A nucleic acid molecule that encodes the fusion protein of claim 17.
29. The nucleic acid molecule of claim 28, wherein the nucleic acid molecule has a nucleotide sequence comprising the nucleotide sequence of SEQ ID NO:53.
30. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion protein, wherein the TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.
31. The pharmaceutical composition of claim 30, wherein the TACI-immunoglobulin fusion protein is a dimer.
32. The use of a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion protein for the manufacture a medicament for reducing circulating blood levels of ZTNF4 in a mammalian subject, wherein the TACI-immunoglobulin fusion protein comprises:
- (a) a TACI receptor moiety that consists of a fragment of a polypeptide that has the amino acid sequence of amino acid residues 30 to 154 of SEQ ID NO:2, wherein the TACI receptor moiety comprises at least one of (i) amino acid residues 34 to 66 of SEQ ID NO:2, and (ii) amino acid residues 71 to 104 of SEQ ID NO:2, and wherein the TACI receptor moiety binds ZTNF4, and
 - (b) an immunoglobulin moiety comprising a constant region of an immunoglobulin.
33. The use of claim 32, wherein the TACI receptor moiety comprises amino acid residues 34 to 66 of SEQ ID NO:2, and amino acid residues 71 to 104 of SEQ ID NO:2.
34. The use of claim 32, wherein the TACI receptor moiety comprises amino acid residues 34 to 104 of SEQ ID NO:2.
35. The use of claim 32, wherein the TACI receptor moiety has the amino acid sequence of amino acid residues 30 to 110 of SEQ ID NO:2

36. The use of claim 32, wherein the immunoglobulin moiety comprises a heavy chain constant region.

37. The use of claim 36, wherein the immunoglobulin moiety comprises a human heavy chain constant region.

38. The use of claim 37, wherein the heavy chain constant region is an IgG1 heavy chain constant region.

39. The use of claim 38, wherein the immunoglobulin moiety is an IgG1 Fc fragment that comprises C_{H2} and C_{H3} domains.

40. The use of claim 39, wherein the immunoglobulin moiety is an IgG1 Fc fragment comprising the amino acid sequence of SEQ ID NO:33.

41. The use of claim 40, wherein TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

42. The use of claim 32, wherein the TACI-immunoglobulin fusion protein is a dimer.

43. The use of a transmembrane activator and calcium modulator and cyclophilin ligand-interactor (TACI)-immunoglobulin fusion protein for the manufacture a medicament, wherein the TACI-immunoglobulin fusion protein has an amino acid sequence comprising the amino acid sequence of SEQ ID NO:54.

44. The use of claim 43, wherein the TACI-immunoglobulin fusion protein is a dimer.

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MSG LGRSRRG GRSRVDQEER FPQGLWTGVA MRS CPEEQYW DPLLGT C MSC

60 70 80 90 100
KTICNHQSQR TCAAFQ RSL S CRKEQGKFYD HLLRDCISCA SIGGQH PKQC

110 120 130 140 150
AYECENKLRS PVNLPPELRR QRSGEVENNS DNSGRYQGLE HRGSEAS PAL

160 170 180 190 200
PGLKLSADQV ALVYST LGLC LCAVLCCFLV AVACFL KKR G DPCSCQPRSR

210 220 230 240 250
PRQSPAKSSQ DHAMEAGSPV STSPEPVETC SFCFPECRAP TQESAVTPGT

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Figure 1

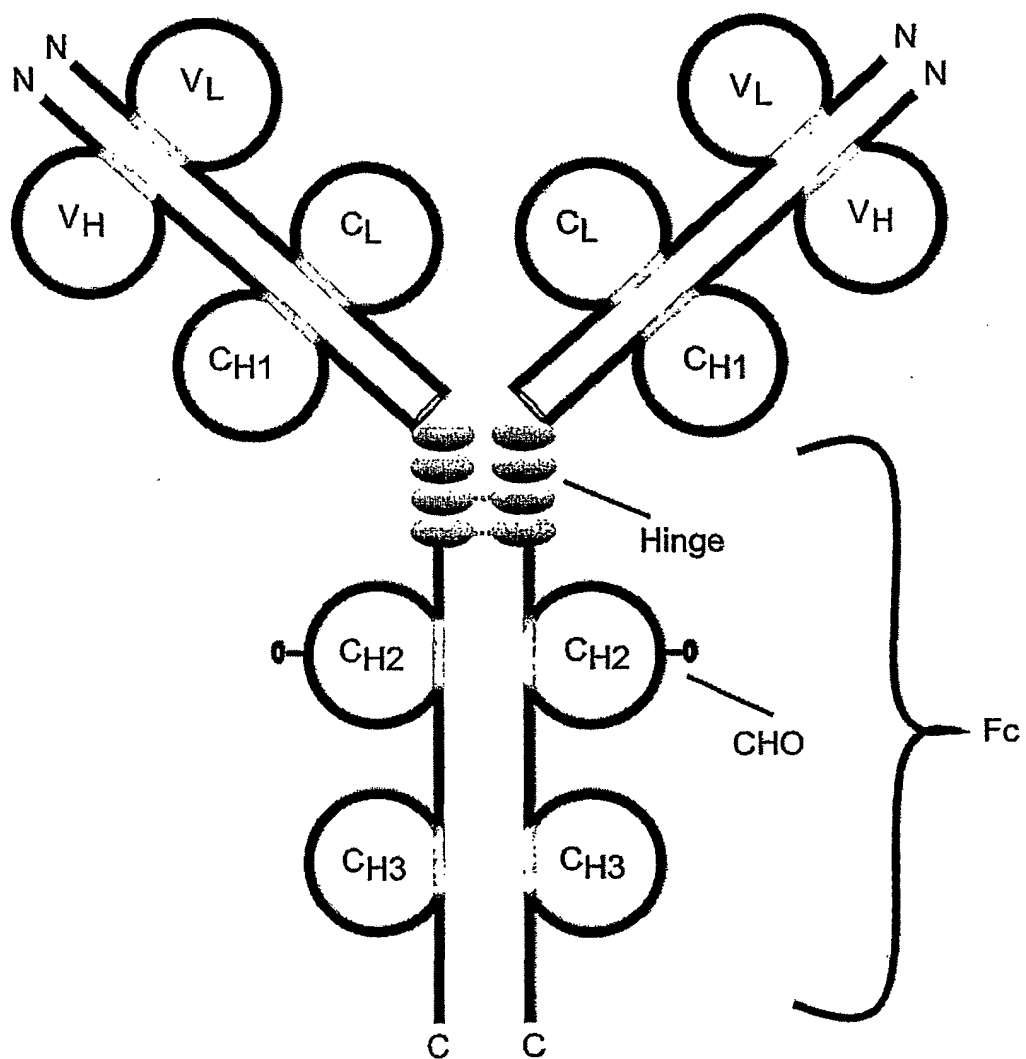


Figure 2

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Figure 3A

[illegible][illegible][illegible][illegible]

Figure 3B

																	350
wt	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr		
Fc-488
Fc4
Fc5
Fc6
Fc7
Fc8
						<- CH2	CH3	->									

																	356	358		365
wt	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu					
Fc-488
Fc4
Fc5
Fc6
Fc7
Fc8

																				380
wt	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu					
Fc-488
Fc4
Fc5
Fc6
Fc7
Fc8

																				395
wt	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro					
Fc-488
Fc4
Fc5
Fc6
Fc7
Fc8

Figure 3C

[illegible][illegible][illegible]

						446		
wt	Leu	Ser	Leu	Ser	Pro	Gly	Lys	***
Fc-488
Fc4
Fc5
Fc6	***	.
Fc7
Fc8

Figure 3D

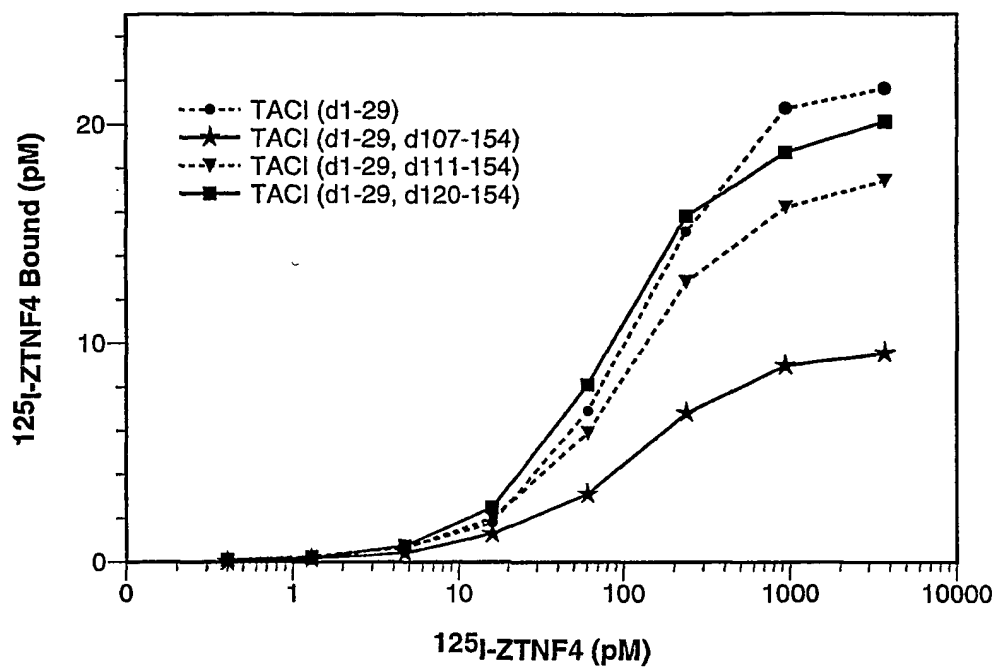


Figure 4

SEQUENCE LISTING

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      15              20              25

gtg gct atg aga tcc tgc ccc gaa gag cag tac tgg gat cct ctg ctg     145
Val Ala Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu
      30              35              40

ggg acc tgc atg tcc tgc aaa acc att tgc aac cat cag agc cag cgc     193
Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg
      45              50              55              60

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acc tgt gca gcc ttc tgc agg tca ctc agc tgc cgc aag gag caa ggc	241
Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln Gly	
65 70 75	
aag ttc tat gac cat ctc ctg agg gac tgc atc agc tgt gcc tcc atc	289
Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala Ser Ile	
80 85 90	
tgt gga cag cac cct aag caa tgt gca tac ttc tgt gag aac aag ctc	337
Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn Lys Leu	
95 100 105	
agg agc cca gtg aac ctt cca cca gag ctc agg aga cag cgg agt gga	385
Arg Ser Pro Val Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg Ser Gly	
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Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu Glu	
125 130 135 140	
cac aga ggc tca gaa gca agt cca gct ctc ccg ggg ctg aag ctg agt	481
His Arg Gly Ser Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu Ser	
145 150 155	
gca gat cag gtg gcc ctg gtc tac agc acg ctg ggg ctc tgc ctg tgt	529
Ala Asp Gln Val Ala Leu Val Tyr Ser Thr Leu Gly Leu Cys Leu Cys	
160 165 170	
gcc gtc ctc tgc tgc ttc ctg gtg gcg gtg gcc tgc ttc ctc aag aag	577
Ala Val Leu Cys Cys Phe Leu Val Ala Val Ala Cys Phe Leu Lys Lys	
175 180 185	
agg ggg gat ccc tgc tcc tgc cag ccc cgc tca agg ccc cgt caa agt	625
Arg Gly Asp Pro Cys Ser Cys Gln Pro Arg Ser Arg Pro Arg Gln Ser	
190 195 200	
ccg gcc aag tct tcc cag gat cac gcg atg gaa gcc ggc agc cct gtg	673
Pro Ala Lys Ser Ser Gln Asp His Ala Met Glu Ala Gly Ser Pro Val	
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agc aca tcc ccc gag cca gtg gag acc tgc agc ttc tgc ttc cct gag	721
Ser Thr Ser Pro Glu Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu	
225 230 235	

tgc agg gcg ccc acg cag gag agc gca gtc acg cct ggg acc ccc gac 769
 Cys Arg Ala Pro Thr Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp
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 35 40 45
 Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg Thr Cys Ala Ala
 50 55 60
 Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp
 65 70 75 80

His Leu Leu Arg Asp Cys Ile Ser Cys Ala Ser Ile Cys Gly Gln His
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 Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn Lys Leu Arg Ser Pro Val
 100 105 110
 Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg Ser Gly Glu Val Glu Asn
 115 120 125
 Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly Leu Glu His Arg Gly Ser
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 Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys Leu Ser Ala Asp Gln Val
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 Ala Leu Val Tyr Ser Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys
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 Cys Ser Cys Gln Pro Arg Ser Arg Pro Arg Gln Ser Pro Ala Lys Ser
 195 200 205
 Ser Gln Asp His Ala Met Glu Ala Gly Ser Pro Val Ser Thr Ser Pro
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 Glu Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu Cys Arg Ala Pro
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 Thr Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp Pro Thr Cys Ala
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 35 40 45

Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
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 Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
 85 90 95
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 115 120 125
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 Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
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 Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
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 35 40 45
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 50 55 60
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 Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala Leu Glu Ala Trp Glu Asn
 85 90 95
 Gly Glu Arg Ser Arg Lys Arg Arg Ala Val Leu Thr Gln Lys Gln Lys
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 Asn Ser Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile
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Arg	Trp	Val	Leu	Ser	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	
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cca	ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	144
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	
				35					40					45		
ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	192
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
			50					55					60			
gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	240
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
		65					70					75				
ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	288
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
	80					85					90					
ccg	cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	336
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
95					100					105					110	
acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	384
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
				115					120					125		
gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	432
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
			130					135					140			
gcc	aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	480
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
		145					150					155				
cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	528
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	
	160					165					170					
ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	576

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 175 180 185 190
 ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc 624
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 195 200 205
 tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag 672
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 210 215 220
 cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac 720
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 225 230 235
 cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga 762
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 240 245 250

<210> 6
 <211> 251
 <212> PRT
 <213> Homo Sapiens

<400> 6
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 Val Leu Ser Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 20 25 30
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 35 40 45
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 50 55 60
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 65 70 75 80
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 85 90 95
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 100 105 110
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 115 120 125

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 130 135 140
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 145 150 155 160
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 165 170 175
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 180 185 190
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 195 200 205
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 210 215 220
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 7
 <211> 44
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> PCR primer.

<400> 7
 atcagcggaa ttcagatctt cagacaaaac tcacacatgc ccac

44

<210> 8
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> PCR primer.

<400> 8
 ggcagtctct agatcattta cccggagaca gggag

35

<210> 9
 <211> 51
 <212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 9

ccgtgcccag cacctgaagc cgagggggca ccgtcagtct tcctcttccc c 51

<210> 10

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 10

ggattctaga ttatttaccg ggagacaggg a. 31

<210> 11

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 11

ggtggcggct cccagatggg tcctgtccga gccagatct tcagacaaaa ctcac 55

<210> 12

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 12

tgggagggct ttgttgga 18

<210> 13

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 13

tccaacaaag ccctcccatc ctccatcgag aaaaccatct cc 42

<210> 14

<211> 57

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 14

ggatggatcc atgaagcacc tgtggttctt ctcctgctg gtggcggctc ccagatg 57

<210> 15

<211> 59

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 15

ctcagccagg aaatccatgc cgagttgaga cgcttccgta gaatgagtgg cctgggccc 59

<210> 16

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 16

gcatgtgtga gttttgtctg aagatctggg ctccttcagc cccgggag 48

<210> 17

<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 17
gcacagaggc tcagaagcaa gtccagctct cccggggctg aaggagccca gatcttcaga 60

<210> 18
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 18
ggggtgggta caacccaga gctgttttaa tctagattat ttacccggag acaggg 56

<210> 19
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 19
gagcccaaatt cttcagacaa aactcacaca tgccca 36

<210> 20
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 20
taattggcgc gcctctagat tatttaccg gagaca 36

<210> 21
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 21
ggcgcgcctc tagattaacc cggagacagg gagaggc 37

<210> 22
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 22
gagcccaaatt cttgcgacaa aactcaca 28

<210> 23
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 23
gtacgtgctt tggtagctgct cctcccgcgg ctt 33

<210> 24
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 24
cagtaccaa gcacgtaccg tgtggtca 28

<210> 25
 <211> 35
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Optimized tPA leader.

<400> 25
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 1 5 10 15
 Ala Val Phe Val Ser Leu Ser Gln Glu Ile His Ala Glu Leu Arg Arg
 20 25 30
 Phe Arg Arg
 35

<210> 26
 <211> 995
 <212> DNA
 <213> Homo sapiens

<220>
 <221> CDS
 <222> (219)...(770)

<400> 26
 aagactcaaa cttagaaact tgaattagat gtggtattca aatccttacg tgccgcgaag 60
 acacagacag cccccgtaag aaccacgaa gcaggcgaag ttcattgttc tcaacattct 120
 agctgctctt gctgcatttg ctctggaatt cttgtagaga tattacttgt ccttccaggc 180
 tgttctttct gtagctccct tgttttcttt ttgtgatc atg ttg cag atg gct ggg 236
 Met Leu Gln Met Ala Gly
 1 5

cag tgc tcc caa aat gaa tat ttt gac agt ttg ttg cat gct tgc ata 284
 Gln Cys Ser Gln Asn Glu Tyr Phe Asp Ser Leu Leu His Ala Cys Ile
 10 15 20

cct tgt caa ctt cga tgt tct tct aat act cct cct cta aca tgt cag 332
 Pro Cys Gln Leu Arg Cys Ser Ser Asn Thr Pro Pro Leu Thr Cys Gln
 25 30 35

cgt tat tgt aat gca agt gtg acc aat tca gtg aaa gga acg aat gcg 380
 Arg Tyr Cys Asn Ala Ser Val Thr Asn Ser Val Lys Gly Thr Asn Ala
 40 45 50

att ctc tgg acc tgt ttg gga ctg agc tta ata att tct ttg gca gtt 428
 Ile Leu Trp Thr Cys Leu Gly Leu Ser Leu Ile Ile Ser Leu Ala Val
 55 60 65 70

ttc gtg cta atg ttt ttg cta agg aag ata agc tct gaa cca tta aag 476
 Phe Val Leu Met Phe Leu Leu Arg Lys Ile Ser Ser Glu Pro Leu Lys
 75 80 85

gac gag ttt aaa aac aca gga tca ggt ctc ctg ggc atg gct aac att 524
 Asp Glu Phe Lys Asn Thr Gly Ser Gly Leu Leu Gly Met Ala Asn Ile
 90 95 100

gac ctg gaa aag agc agg act ggt gat gaa att att ctt ccg aga ggc 572
 Asp Leu Glu Lys Ser Arg Thr Gly Asp Glu Ile Ile Leu Pro Arg Gly
 105 110 115

ctc gag tac acg gtg gaa gaa tgc acc tgt gaa gac tgc atc aag agc 620
 Leu Glu Tyr Thr Val Glu Glu Cys Thr Cys Glu Asp Cys Ile Lys Ser
 120 125 130

aaa ccg aag gtc gac tct gac cat tgc ttt cca ctc cca gct atg gag 668
 Lys Pro Lys Val Asp Ser Asp His Cys Phe Pro Leu Pro Ala Met Glu
 135 140 145 150

gaa ggc gca acc att ctt gtc acc acg aaa acg aat gac tat tgc aag 716
 Glu Gly Ala Thr Ile Leu Val Thr Thr Lys Thr Asn Asp Tyr Cys Lys
 155 160 165

agc ctg cca gct gct ttg agt gct acg gag ata gag aaa tca att tct 764
 Ser Leu Pro Ala Ala Leu Ser Ala Thr Glu Ile Glu Lys Ser Ile Ser
 170 175 180

gct agg taattaacca ttctgactcg agcagtgcca ctttaaaaat cttttgtcag 820
 Ala Arg

aatagatgat gtgtcagatc tctttaggat gactgtatatt ttcagttgcc gatacagctt 880
 tttgtcctct aactgtggaa actctttatg ttagatatat ttctctaggt tactgttggg 940
 agcttaatgg tagaaacttc cttggtttca tgattaaagt cttttttttt cctga 995

<210> 27
 <211> 184
 <212> PRT
 <213> Homo sapiens

<400> 27
 Met Leu Gln Met Ala Gly Gln Cys Ser Gln Asn Glu Tyr Phe Asp Ser
 1 5 10 15
 Leu Leu His Ala Cys Ile Pro Cys Gln Leu Arg Cys Ser Ser Asn Thr
 20 25 30
 Pro Pro Leu Thr Cys Gln Arg Tyr Cys Asn Ala Ser Val Thr Asn Ser
 35 40 45
 Val Lys Gly Thr Asn Ala Ile Leu Trp Thr Cys Leu Gly Leu Ser Leu
 50 55 60
 Ile Ile Ser Leu Ala Val Phe Val Leu Met Phe Leu Leu Arg Lys Ile
 65 70 75 80
 Ser Ser Glu Pro Leu Lys Asp Glu Phe Lys Asn Thr Gly Ser Gly Leu
 85 90 95
 Leu Gly Met Ala Asn Ile Asp Leu Glu Lys Ser Arg Thr Gly Asp Glu
 100 105 110
 Ile Ile Leu Pro Arg Gly Leu Glu Tyr Thr Val Glu Glu Cys Thr Cys
 115 120 125
 Glu Asp Cys Ile Lys Ser Lys Pro Lys Val Asp Ser Asp His Cys Phe
 130 135 140
 Pro Leu Pro Ala Met Glu Glu Gly Ala Thr Ile Leu Val Thr Thr Lys
 145 150 155 160
 Thr Asn Asp Tyr Cys Lys Ser Leu Pro Ala Ala Leu Ser Ala Thr Glu
 165 170 175
 Ile Glu Lys Ser Ile Ser Ala Arg
 180

<210> 28
 <211> 762
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Modified immunoglobulin moiety.

<221> CDS
 <222> (7)...(759)

<400> 28

ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc	48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro	
1 5 10	
aga tgg gtc ctg tcc gag ccc aga tct tca gac aaa act cac aca tgc	96
Arg Trp Val Leu Ser Glu Pro Arg Ser Ser Asp Lys Thr His Thr Cys	
15 20 25 30	
cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc	144
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu	
35 40 45	
ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag	192
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu	
50 55 60	
gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag	240
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys	
65 70 75	
ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag	288
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys	
80 85 90	
ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc	336
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu	
95 100 105 110	
acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag	384
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys	
115 120 125	
gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa	432
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys	
130 135 140	
gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc	480
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser	
145 150 155	
cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa	528
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys	
160 165 170	

ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 576
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 175 180 185 190

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc 624
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 195 200 205

tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag 672
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 210 215 220

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac 720
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 225 230 235

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga 762
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 240 245 250

<210> 29

<211> 251

<212> PRT

<213> Artificial Sequence

<400> 29

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 Val Leu Ser Glu Pro Arg Ser Ser Asp Lys Thr His Thr Cys Pro Pro
 20 25 30
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 35 40 45
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 50 55 60
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 65 70 75 80
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 85 90 95
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 100 105 110
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 115 120 125

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 130 135 140
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 145 150 155 160
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 165 170 175
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 180 185 190
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 195 200 205
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 210 215 220
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 30
 <211> 762
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Modified immunoglobulin moiety.

<221> CDS
 <222> (7)...(759)

<400> 30

ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc 48
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro

1 5 10

aga tgg gtc ctg tcc gag ccc aga tct tca gac aaa act cac aca tgc 96
 Arg Trp Val Leu Ser Glu Pro Arg Ser Ser Asp Lys Thr His Thr Cys
 15 20 25 30

cca ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc 144
 Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu
 35 40 45

ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag 192

Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
			50					55					60			
gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	240
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
		65					70					75				
ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	288
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
	80					85				90						
ccg	cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	336
Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
	95				100					105					110	
acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	384
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
			115					120						125		
gtc	tcc	aac	aaa	gcc	ctc	cca	tcc	tcc	atc	gag	aaa	acc	atc	tcc	aaa	432
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
			130				135						140			
gcc	aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	480
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
		145					150					155				
cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	528
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	
	160					165				170						
ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	576
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	
	175				180				185						190	
ccg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	tcc	gac	ggc	624
Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	
				195				200					205			
tcc	ttc	ttc	ctc	tac	agc	aag	ctc	acc	gtg	gac	aag	agc	agg	tgg	cag	672
Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	
			210					215					220			

<400> 31															
Met	Lys	His	Leu	Trp	Phe	Phe	Leu	Leu	Leu	Val	Ala	Ala	Pro	Arg	Trp
1				5					10					15	
Val	Leu	Ser	Glu	Pro	Arg	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro
			20					25					30		
Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu	Phe	Pro
		35					40					45			
Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr
	50					55					60				
Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
65				70						75					80
Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg
			85						90					95	
Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val
			100					105					110		
Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser
		115					120					125			
Asn	Lys	Ala	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys
	130					135					140				
Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp
145				150						155					160
Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe
			165					170						175	
Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu
			180					185					190		
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe
		195					200					205			
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly
	210					215					220				

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 32
 <211> 762
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Modified immunoglobulin moiety.

<221> CDS
 <222> (7)...(759)

<400> 32
 ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc 48
 Met Lys His Leu Trp Phe Phe Leu Leu Val Ala Ala Pro
 1 5 10
 aga tgg gtc ctg tcc gag ccc aaa tct tca gac aaa act cac aca tgc 96
 Arg Trp Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys
 15 20 25 30
 cca ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc 144
 Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu
 35 40 45
 ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag 192
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 50 55 60
 gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag 240
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
 65 70 75
 ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag 288
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 80 85 90
 ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc 336

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Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
 95                               100                               105                               110

acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag   384
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
                               115                               120                               125

gtc tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa   432
Val Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys
                               130                               135                               140

gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc   480
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
                               145                               150                               155

cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa   528
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
                               160                               165                               170

ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag   576
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
175                               180                               185                               190

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc   624
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
                               195                               200                               205

tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag   672
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
                               210                               215                               220

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac   720
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
                               225                               230                               235

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga           762
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                               240                               245                               250

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<210> 33

<211> 251

<212> PRT

<213> Artificial Sequence

<400> 33

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Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1           5           10           15
Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
           20           25           30
Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
           35           40           45
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
           50           55           60
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
65           70           75           80
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
           85           90           95
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
           100          105          110
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
           115          120          125
Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
           130          135          140
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
145          150          155          160
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
           165          170          175
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
           180          185          190
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
           195          200          205
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
           210          215          220
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
225          230          235          240
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
           245          250

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<210> 34

<211> 759

<212> DNA

<213> Artificial Sequence

<220>

<223> Modified immunoglobulin moiety.

<221> CDS

<222> (7)...(756)

<400> 34

ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc	48
Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro	
1 5 10	
aga tgg gtc ctg tcc gag ccc aaa tct tca gac aaa act cac aca tgc	96
Arg Trp Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys	
15 20 25 30	
cca ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc	144
Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu	
35 40 45	
ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag	192
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu	
50 55 60	
gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag	240
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys	
65 70 75	
ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag	288
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys	
80 85 90	
ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc	336
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu	
95 100 105 110	
acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag	384
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys	
115 120 125	
gtc tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa	432
Val Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys	
130 135 140	
gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc	480

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 145 150 155

cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa 528
 Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 160 165 170

ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag 576
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 175 180 185 190

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc 624
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 195 200 205

tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag 672
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 210 215 220

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac 720
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 225 230 235

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt tga 759
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 240 245 250

<210> 35

<211> 250

<212> PRT

<213> Artificial Sequence

<400> 35

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15

Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
 20 25 30

Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro
 35 40 45

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 50 55 60

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 65 70 75 80
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 85 90 95
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 100 105 110
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 115 120 125
 Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
 130 135 140
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 145 150 155 160
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 165 170 175
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 180 185 190
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 195 200 205
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 210 215 220
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 245 250

<210> 36

<211> 762

<212> DNA

<213> Artificial Sequence

<220>

<223> Modified immunoglobulin moiety.

<221> CDS

<222> (7)...(759)

<400> 36

ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc 48
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro
 1 5 10

aga tgg gtc ctg tcc gag ccc aaa tct tgc gac aaa act cac aca tgc 96

Arg	Trp	Val	Leu	Ser	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	
15					20					25					30	
cca	ccg	tgc	cca	gca	cct	gaa	ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	144
Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	
				35					40					45		
ttc	ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	192
Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	
			50					55					60			
gtc	aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	240
Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	
		65					70					75				
ttc	aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	288
Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
	80					85					90					
ccg	cgg	gag	gag	cag	tac	caa	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	336
Pro	Arg	Glu	Glu	Gln	Tyr	Gln	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	
	95				100					105					110	
acc	gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	384
Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	
				115					120					125		
gtc	tcc	aac	aaa	gcc	ctc	cca	gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	432
Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	
			130					135					140			
gcc	aaa	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg	ccc	cca	tcc	480
Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	
		145					150					155				
cgg	gat	gag	ctg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	ctg	gtc	aaa	528
Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	
	160					165				170						
ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	aat	ggg	cag	576
Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	
175					180				185						190	

ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc 624
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
 195 200 205

tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag 672
 Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 210 215 220

cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac 720
 Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 225 230 235

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga 762
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 240 245 250

<210> 37

<211> 251

<212> PRT

<213> Artificial Sequence

<400> 37

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 Val Leu Ser Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro
 20 25 30
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 35 40 45
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 50 55 60
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 65 70 75 80
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 85 90 95
 Glu Glu Gln Tyr Gln Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 100 105 110
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 115 120 125
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 130 135 140
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 145 150 155 160

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 165 170 175
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 180 185 190
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 195 200 205
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 210 215 220
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 38

<211> 762

<212> DNA

<213> Artificial Sequence

<220>

<223> Modified immunoglobulin moiety.

<221> CDS

<222> (7)...(759)

<400> 38

ggatcc atg aag cac ctg tgg ttc ttc ctc ctg ctg gtg gcg gct ccc 48
 Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro
 1 5 10

aga tgg gtc ctg tcc gag ccc aaa tct tca gac aaa act cac aca tgc 96
 Arg Trp Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys
 15 20 25 30

cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc 144
 Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
 35 40 45

ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag 192
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 50 55 60

gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag 240

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys	
65 70 75	
ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag	288
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys	
80 85 90	
ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc	336
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu	
95 100 105 110	
acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag	384
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys	
115 120 125	
gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa	432
Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys	
130 135 140	
gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc	480
Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser	
145 150 155	
cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa	528
Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys	
160 165 170	
ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag	576
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln	
175 180 185 190	
ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc	624
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly	
195 200 205	
tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag	672
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln	
210 215 220	
cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac	720
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn	
225 230 235	

cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa tga
 His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 240 245 250

762

<210> 39

<211> 251

<212> PRT

<213> Artificial Sequence

<400> 39

Met Lys His Leu Trp Phe Phe Leu Leu Leu Val Ala Ala Pro Arg Trp
 1 5 10 15
 Val Leu Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
 20 25 30
 Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
 35 40 45
 Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
 50 55 60
 Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
 65 70 75 80
 Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
 85 90 95
 Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
 100 105 110
 Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
 115 120 125
 Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
 130 135 140
 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
 145 150 155 160
 Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
 165 170 175
 Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
 180 185 190
 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
 195 200 205
 Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
 210 215 220
 Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
 225 230 235 240
 Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 245 250

<210> 40
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 40
tattaggccg gccacatgg atgcaatga 29

<210> 41
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 41
tgaagatttg ggctccttga gacctggga 29

<210> 42
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 42
tcccaggctc caaggagccc aaatcttca 29

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 43
tgaagatttg ggctcggttct cacagaagta 30

<210> 44
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 44
atacttctgt gagaacgagc ccaaattcttc a 31

<210> 45
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 45
tttgggctcg ctctgagct tgttctcaca 30

<210> 46
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 46
ctcaggagcg agcccaaattc ttcagaca 28

<210> 47
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer.

<400> 47

tttgggctcc ctgagctctg gtggaa

26

<210> 48

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> PCR primer.

<400> 48

gagctcaggg agcccaaattc ttccagaca

28

<210> 49

<211> 1214

<212> DNA

<213> Artificial Sequence

<220>

<223> Fusion protein.

<221> CDS

<222> (17)...(1192)

<400> 49

tattaggccg gccacc atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg 52

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu

1

5

10

ctg ctg tgt ggc gcc gtc ttc gtt tgc ctc agc cag gaa atc cat gcc 100

Leu Leu Cys Gly Ala Val Phe Val Ser Leu Ser Gln Glu Ile His Ala

15

20

25

gag ttg aga cgc ttc cgt aga gct atg aga tcc tgc ccc gaa gag cag 148

Glu Leu Arg Arg Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln

30

35

40

tac tgg gat cct ctg ctg ggt acc tgc atg tcc tgc aaa acc att tgc 196

Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys

45

50

55

60

aac cat cag agc cag cgc acc tgt gca gcc ttc tgc agg tca ctc agc 244

Asn His Gln Ser Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser	
65 70 75	
tgc cgc aag gag caa ggc aag ttc tat gac cat ctc ctg agg gac tgc	292
Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys	
80 85 90	
atc agc tgt gcc tcc atc tgt gga cag cac cct aag caa tgt gca tac	340
Ile Ser Cys Ala Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr	
95 100 105	
ttc tgt gag aac aag ctc agg agc cca gtg aac ctt cca cca gag ctc	388
Phe Cys Glu Asn Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu	
110 115 120	
agg aga cag cgg agt gga gaa gtt gaa aac aat tca gac aac tcg gga	436
Arg Arg Gln Arg Ser Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly	
125 130 135 140	
agg tac caa gga ttg gag cac aga ggc tca gaa gca agt cca gct ctc	484
Arg Tyr Gln Gly Leu Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu	
145 150 155	
cca ggt ctc aag gag ccc aaa tct tca gac aaa act cac aca tgc cca	532
Pro Gly Leu Lys Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro	
160 165 170	
ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc ttc	580
Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe	
175 180 185	
ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc	628
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val	
190 195 200	
aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc	676
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe	
205 210 215 220	
aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg	724
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro	
225 230 235	

cgg gag gag cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc	772
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr	
240 245 250	
gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc	820
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val	
255 260 265	
tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa gcc	868
Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala	
270 275 280	
aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg	916
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg	
285 290 295 300	
gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc	964
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly	
305 310 315	
ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg	1012
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro	
320 325 330	
gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc	1060
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser	
335 340 345	
ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag	1108
Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln	
350 355 360	
ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac	1156
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His	
365 370 375 380	
tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taatctagag	1202
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385 390	
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 <213> Artificial Sequence

<220>
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 35 40 45
 Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser
 50 55 60
 Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu
 65 70 75 80
 Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
 85 90 95
 Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn
 100 105 110
 Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu Arg Arg Gln Arg
 115 120 125
 Ser Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg Tyr Gln Gly
 130 135 140
 Leu Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu Pro Gly Leu Lys
 145 150 155 160
 Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 165 170 175
 Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 180 185 190
 Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 195 200 205
 Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 210 215 220
 Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 225 230 235 240
 Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 245 250 255
 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
 260 265 270

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 275 280 285
 Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 290 295 300
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
 305 310 315 320
 Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 325 330 335
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 340 345 350
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
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 370 375 380
 Ser Leu Ser Leu Ser Pro Gly Lys
 385 390

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 <213> Artificial Sequence

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 Leu Leu Cys Gly Ala Val Phe Val Ser Leu Ser Gln Glu Ile His Ala
 15 20 25

 gag ttg aga cgc ttc cgt aga gct atg aga tcc tgc ccc gaa gag cag 148
 Glu Leu Arg Arg Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln
 30 35 40

 tac tgg gat cct ctg ctg ggt acc tgc atg tcc tgc aaa acc att tgc 196

Tyr	Trp	Asp	Pro	Leu	Leu	Gly	Thr	Cys	Met	Ser	Cys	Lys	Thr	Ile	Cys	
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aac	cat	cag	agc	cag	cgc	acc	tgt	gca	gcc	ttc	tgc	agg	tca	ctc	agc	244
Asn	His	Gln	Ser	Gln	Arg	Thr	Cys	Ala	Ala	Phe	Cys	Arg	Ser	Leu	Ser	
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tgc	cgc	aag	gag	caa	ggc	aag	ttc	tat	gac	cat	ctc	ctg	agg	gac	tgc	292
Cys	Arg	Lys	Glu	Gln	Gly	Lys	Phe	Tyr	Asp	His	Leu	Leu	Arg	Asp	Cys	
			80					85						90		
atc	agc	tgt	gcc	tcc	atc	tgt	gga	cag	cac	cct	aag	caa	tgt	gca	tac	340
Ile	Ser	Cys	Ala	Ser	Ile	Cys	Gly	Gln	His	Pro	Lys	Gln	Cys	Ala	Tyr	
			95				100					105				
ttc	tgt	gag	aac	gag	ccc	aaa	tct	tca	gac	aaa	act	cac	aca	tgc	cca	388
Phe	Cys	Glu	Asn	Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	
	110					115					120					
ccg	tgc	cca	gca	cct	gaa	gcc	gag	ggg	gca	ccg	tca	gtc	ttc	ctc	ttc	436
Pro	Cys	Pro	Ala	Pro	Glu	Ala	Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu	Phe	
125					130					135					140	
ccc	cca	aaa	ccc	aag	gac	acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	484
Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
				145					150					155		
aca	tgc	gtg	gtg	gtg	gac	gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	532
Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
			160					165					170			
aac	tgg	tac	gtg	gac	ggc	gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	580
Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
			175				180					185				
cgg	gag	gag	cag	tac	aac	agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	628
Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	
	190					195					200					
gtc	ctg	cac	cag	gac	tgg	ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	676
Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	
205					210					215					220	

tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa gcc 724
 Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
 225 230 235

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg 772
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 240 245 250

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc 820
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 255 260 265

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg 868
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 270 275 280

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc 916
 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 285 290 295 300

ttc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag 964
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 305 310 315

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac 1012
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 320 325 330

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa taatctagag 1058
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 335 340

gcgcgccaat ta 1070

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<211> 344

<212> PRT

<213> Artificial Sequence

<220>

<223> Fusion protein.

<400> 52

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Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro
 35           40           45
Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser
 50           55           60
Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu
 65           70           75           80
Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
 85           90           95
Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn
100          105          110
Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
115          120          125
Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
130          135          140
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
145          150          155          160
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
165          170          175
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
180          185          190
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
195          200          205
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
210          215          220
Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
225          230          235          240
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
245          250          255
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
260          265          270
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
275          280          285
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
290          295          300
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
305          310          315          320
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Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 53

<211> 1082

<212> DNA

<213> Artificial Sequence

<220>

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<221> CDS

<222> (17)...(1060)

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Leu Leu Cys Gly Ala Val Phe Val Ser Leu Ser Gln Glu Ile His Ala
15 20 25

gag ttg aga cgc ttc cgt aga gct atg aga tcc tgc ccc gaa gag cag 148
Glu Leu Arg Arg Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln
30 35 40

tac tgg gat cct ctg ctg ggt acc tgc atg tcc tgc aaa acc att tgc 196
Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys
45 50 55 60

aac cat cag agc cag cgc acc tgt gca gcc ttc tgc agg tca ctc agc 244
Asn His Gln Ser Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser
65 70 75

tgc cgc aag gag caa ggc aag ttc tat gac cat ctc ctg agg gac tgc 292
Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys
80 85 90

atc agc tgt gcc tcc atc tgt gga cag cac cct aag caa tgt gca tac 340
Ile Ser Cys Ala Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr
95 100 105

ttc tgt gag aac aag ctc agg agc gag ccc aaa tct tca gac aaa act	388
Phe Cys Glu Asn Lys Leu Arg Ser Glu Pro Lys Ser Ser Asp Lys Thr	
110 115 120	
cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggg gca ccg tca	436
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser	
125 130 135 140	
gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg	484
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg	
145 150 155	
acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct	532
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro	
160 165 170	
gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc	580
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala	
175 180 185	
aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgt gtg gtc	628
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val	
190 195 200	
agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac	676
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr	
205 210 215 220	
aag tgc aag gtc tcc aac aaa gcc ctc cca tcc tcc atc gag aaa acc	724
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr	
225 230 235	
atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg	772
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	
240 245 250	
ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc	820
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys	
255 260 265	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	868
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	
270 275 280	

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285                290                295                300

tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc 964
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
                305                310                315

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct 1012
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
                320                325                330

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa 1060
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<210> 54
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 <212> PRT
 <213> Artificial Sequence

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 <223> Fusion protein.

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Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro
                35                40                45
Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser
                50                55                60
Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu
65                70                75                80
Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
                85                90                95
Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn
                100                105                110

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Lys Leu Arg Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
 115 120 125
 Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
 130 135 140
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 145 150 155 160
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 165 170 175
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 180 185 190
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 195 200 205
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 210 215 220
 Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
 225 230 235 240
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 245 250 255
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 260 265 270
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 275 280 285
 Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
 290 295 300
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
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 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 340 345

<210> 55

<211> 1109

<212> DNA.

<213> Artificial Sequence

<220>

<223> Fusion protein.

<221> CDS

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 Leu Leu Cys Gly Ala Val Phe Val Ser Leu Ser Gln Glu Ile His Ala
 15 20 25

 gag ttg aga cgc ttc cgt aga gct atg aga tcc tgc ccc gaa gag cag 148
 Glu Leu Arg Arg Phe Arg Arg Ala Met Arg Ser Cys Pro Glu Glu Gln
 30 35 40

 tac tgg gat cct ctg ctg ggt acc tgc atg tcc tgc aaa acc att tgc 196
 Tyr Trp Asp Pro Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys
 45 50 55 60

 aac cat cag agc cag cgc acc tgt gca gcc ttc tgc agg tca ctc agc 244
 Asn His Gln Ser Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser
 65 70 75

 tgc cgc aag gag caa ggc aag ttc tat gac cat ctc ctg agg gac tgc 292
 Cys Arg Lys Glu Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys
 80 85 90

 atc agc tgt gcc tcc atc tgt gga cag cac cct aag caa tgt gca tac 340
 Ile Ser Cys Ala Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr
 95 100 105

 ttc tgt gag aac aag ctc agg agc cca gtg aac ctt cca cca gag ctc 388
 Phe Cys Glu Asn Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu
 110 115 120

 agg gag ccc aaa tct tca gac aaa act cac aca tgc cca ccg tgc cca 436
 Arg Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
 125 130 135 140

 gca cct gaa gcc gag ggg gca ccg tca gtc ttc ctc ttc ccc cca aaa 484
 Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys
 145 150 155

 ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg 532

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	
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gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac	580
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr	
175 180 185	
gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag	628
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu	
190 195 200	
cag tac aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac	676
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His	
205 210 215 220	
cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa	724
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	
225 230 235	
gcc ctc cca tcc tcc atc gag aaa acc atc tcc aaa gcc aaa ggg cag	772
Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln	
240 245 250	
ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg	820
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu	
255 260 265	
acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc	868
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro	
270 275 280	
agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac	916
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn	
285 290 295 300	
tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc	964
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu	
305 310 315	
tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc	1012
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val	
320 325 330	

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag 1060
 Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
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<211> 357

<212> PRT

<213> Artificial Sequence

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			20				25					30			
Phe	Arg	Arg	Ala	Met	Arg	Ser	Cys	Pro	Glu	Glu	Gln	Tyr	Trp	Asp	Pro
	35						40					45			
Leu	Leu	Gly	Thr	Cys	Met	Ser	Cys	Lys	Thr	Ile	Cys	Asn	His	Gln	Ser
	50					55					60				
Gln	Arg	Thr	Cys	Ala	Ala	Phe	Cys	Arg	Ser	Leu	Ser	Cys	Arg	Lys	Glu
65				70					75					80	
Gln	Gly	Lys	Phe	Tyr	Asp	His	Leu	Leu	Arg	Asp	Cys	Ile	Ser	Cys	Ala
			85						90					95	
Ser	Ile	Cys	Gly	Gln	His	Pro	Lys	Gln	Cys	Ala	Tyr	Phe	Cys	Glu	Asn
			100					105						110	
Lys	Leu	Arg	Ser	Pro	Val	Asn	Leu	Pro	Pro	Glu	Leu	Arg	Glu	Pro	Lys
	115						120					125			
Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Ala
	130					135					140				
Glu	Gly	Ala	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr
145				150					155					160	
Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val
				165					170					175	
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<211> 9
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<213> Artificial Sequence

<220>
<223> Illustrative nucleotide sequence.

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<210> 58
<211> 9
<212> DNA
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<400> 58
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<210> 59
 <211> 586
 <212> DNA
 <213> Homo sapiens

<220>
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 <222> (27)...(578)

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 Gly Arg Asp Ala Pro Ala Pro Thr Pro Cys Val Pro Ala Glu Cys Phe
 10 15 20 25

gac ctg ctg gtc cgc cac tgc gtg gcc tgc ggg ctc ctg cgc acg ccg 149
 Asp Leu Leu Val Arg His Cys Val Ala Cys Gly Leu Leu Arg Thr Pro
 30 35 40

cgg ccg aaa ccg gcc ggg gcc agc agc cct gcg ccc agg acg gcg ctg 197
 Arg Pro Lys Pro Ala Gly Ala Ser Ser Pro Ala Pro Arg Thr Ala Leu
 45 50 55

cag ccg cag gag tcg gtg ggc gcg ggg gcc ggc gag gcg gcg ctg ccc 245.
 Gln Pro Gln Glu Ser Val Gly Ala Gly Ala Gly Glu Ala Ala Leu Pro
 60 65 70

ctg ccc ggg ctg ctc ttt ggc gcc ccc gcg ctg ctg ggc ctg gca ctg 293
 Leu Pro Gly Leu Leu Phe Gly Ala Pro Ala Leu Leu Gly Leu Ala Leu
 75 80 85

gtc ctg gcg ctg gtc ctg gtg ggt ctg gtg agc tgg agg ccg cga cag 341
 Val Leu Ala Leu Val Leu Val Gly Leu Val Ser Trp Arg Arg Arg Gln
 90 95 100 105

cgg ccg ctt cgc gcc gcg tcc tcc gca gag gcc ccc gac gga gac aag 389

Arg Arg Leu Arg Gly Ala Ser Ser Ala Glu Ala Pro Asp Gly Asp Lys
 110 115 120

gac gcc cca gag ccc ctg gac aag gtc atc att ctg tct ccg gga atc 437
 Asp Ala Pro Glu Pro Leu Asp Lys Val Ile Ile Leu Ser Pro Gly Ile
 125 130 135

tct gat gcc aca gct cct gcc tgg cct cct cct ggg gaa gac cca gga 485
 Ser Asp Ala Thr Ala Pro Ala Trp Pro Pro Pro Gly Glu Asp Pro Gly
 140 145 150

acc acc cca cct ggc cac agt gtc cct gtg cca gcc aca gag ctg ggc 533
 Thr Thr Pro Pro Gly His Ser Val Pro Val Pro Ala Thr Glu Leu Gly
 155 160 165

tcc act gaa ctg gtg acc acc aag acg gcc ggc cct gag caa caa 578
 Ser Thr Glu Leu Val Thr Thr Lys Thr Ala Gly Pro Glu Gln Gln
 170 175 180

tagcaggg 586

<210> 60
 <211> 184
 <212> PRT
 <213> Homo sapiens

<400> 60
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 1 5 10 15
 Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg His Cys
 20 25 30
 Val Ala Cys Gly Leu Leu Arg Thr Pro Arg Pro Lys Pro Ala Gly Ala
 35 40 45
 Ser Ser Pro Ala Pro Arg Thr Ala Leu Gln Pro Gln Glu Ser Val Gly
 50 55 60
 Ala Gly Ala Gly Glu Ala Ala Leu Pro Leu Pro Gly Leu Leu Phe Gly
 65 70 75 80
 Ala Pro Ala Leu Leu Gly Leu Ala Leu Val Leu Ala Leu Val Leu Val
 85 90 95
 Gly Leu Val Ser Trp Arg Arg Arg Gln Arg Arg Leu Arg Gly Ala Ser
 100 105 110

Ser Ala Glu Ala Pro Asp Gly Asp Lys Asp Ala Pro Glu Pro Leu Asp
 115 120 125
 Lys Val Ile Ile Leu Ser Pro Gly Ile Ser Asp Ala Thr Ala Pro Ala
 130 135 140
 Trp Pro Pro Pro Gly Glu Asp Pro Gly Thr Thr Pro Pro Gly His Ser
 145 150 155 160
 Val Pro Val Pro Ala Thr Glu Leu Gly Ser Thr Glu Leu Val Thr Thr
 165 170 175
 Lys Thr Ala Gly Pro Glu Gln Gln
 180

<210> 61

<211> 19

<212> PRT

<213> Artificial Sequence

<220>

<223> 26-10 VH signal sequence.

<400> 61

Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val Leu Ser

<210> 62

<211> 332

<212> PRT

<213> Artificial Sequence

<220>

<223> Fusion protein.

<400> 62

Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
 1 5 10 15
 Val Leu Ser Ala Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro
 20 25 30
 Leu Leu Gly Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser
 35 40 45
 Gln Arg Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu
 50 55 60

Gln Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
 65 70 75 80
 Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu Asn
 85 90 95
 Lys Leu Arg Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro
 100 105 110
 Pro Cys Pro Ala Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe
 115 120 125
 Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
 130 135 140
 Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
 145 150 155 160
 Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
 165 170 175
 Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
 180 185 190
 Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
 195 200 205
 Ser Asn Lys Ala Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala
 210 215 220
 Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
 225 230 235 240
 Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
 245 250 255
 Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
 260 265 270
 Glu Asn Asn Tyr Lys Thr Thr Pro Val Leu Asp Ser Asp Gly Ser
 275 280 285
 Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
 290 295 300
 Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
 305 310 315 320
 Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 325 330

<210> 63

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> 26-10 VH 5' UTR.

<400> 63
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<210> 64
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Modified 26-10 VH 5' UTR.

<400> 64
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<210> 65
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> 26-10 VH signal sequence.

<221> CDS
<222> (1)...(57)

<400> 65
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Met Gly Trp Ser Trp Ile Phe Leu Phe Leu Leu Ser Gly Thr Ala Gly
1 5 10 15

gtc ctc tct 57
Val Leu Ser

<210> 66
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> 26-10 VH intron.

<400> 66

gtaaggggct cccagttcc aaaatctgaa gaaaagaaat ggcttgggat gtcacagata 60
tccactctgt ctttctcttc acag 84

<210> 67

<211> 1135

<212> DNA

<213> Artificial Sequence

<220>

<223> Fusion protein.

<400> 67

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agctggatct ttctctttct tctgtcagga actgcaggta aggggctccc cagttccaaa 120
atctgaagaa aagaaatggc ttgggatgtc acagatatcc actctgtctt tctcttcaca 180
gggtgcctct ctgctatgag atcctgcccc gaagagcagt actgggatcc tctgtctgggt 240
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tgcaggtcac tcagctgccg caaggagcaa ggcaagttct atgaccatct cctgagggac 360
tgcacagct gtgcctccat ctgtggacag caccctaagc aatgtgcata cttctgtgag 420
aacaagctca ggagcgagcc caaatcttca gacaaaactc acacatgccc accgtgccc 480
gcacctgaag ccgagggggc accgtcagtc ttctcttcc ccccaaaacc caaggacacc 540
ctcatgatct cccggacccc tgaggtcaca tgcgtgggtg tggacgtgag ccacgaagac 600
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ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 720
caggactggc tgaatggcaa ggagtacaag tgcaaggctc ccaacaaagc cctcccatcc 780
tccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 840
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ggcttctatc ccagcgacat cgccgtggag tgggagagca atgggcagcc ggagaacaac 960
tacaagacca cgcctcccgt gctggactcc gacggctcct tcttctctta cagcaagctc 1020
accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1080
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa ataaa 1135

<210> 68

<211> 1135

<212> DNA

<213> Artificial Sequence

<220>

<223> Fusion protein.

<400> 68

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agctggatct ttctctttct tctgtcagga actgcaggta aggggctccc cagttccaaa 120


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atctgaagaa aagaaatggc ttgggatgtc acagatatcc actctgtctt tctcttcaca 180
ggtgtcctct ctgctatgag atcctgcccc gaagagcagt actgggatcc tctgctgggt 240
acctgcatgt cctgcaaaac catttgcaac catcagagcc agcgcacctg tgcagccttc 300
tgcaggtcac tcagctgccg caaggagcaa ggcaagtctt atgaccatct cctgagggac 360
tgcacagct gtgctccat ctgtggacag caccctaagc aatgtgcata cttctgtgag 420
aacaagctca ggagcgagcc caaatcttca gacaaaactc acacatgccc accgtgcccc 480
gcacctgaag ccgagggggc accgtcagtc ttctctttcc ccccaaaacc caaggacacc 540
ctcatgatct cccggacccc tgaggtcaca tgcgtggtgg tggacgtgag ccacgaagac 600
cctgaggtca agttcaactg gtacgtggac ggcgtggagg tgcataatgc caagacaaag 660
ccgcgggagg agcagtacaa cagcacgtac cgtgtggtca gcgtcctcac cgtcctgcac 720
caggactggc tgaatggcaa ggagtacaag tgcaaggtct ccaacaaagc cctcccatcc 780
tccatcgaga aaaccatctc caaagccaaa gggcagcccc gagaaccaca ggtgtacacc 840
ctgcccccat cccgggatga gctgaccaag aaccagggtc gcctgacctg cctggtcaaa 900
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accgtggaca agagcaggtg gcagcagggg aacgtcttct catgctccgt gatgcatgag 1080
gctctgcaca accactacac gcagaagagc ctctccctgt ctccgggtaa ataaa 1135

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<210> 69

<211> 884

<212> DNA

<213> Artificial Sequence

<220>

<223> CMV enhancer/MPSV LTR promoter construct.

<400> 69

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cgcgttacat aacttacggt aaatggcccc cctggctgac cgcccaacga ccccgccca 60
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caatgggtgg agtatttacg gtaaaactgcc cacttggcag tacatcaagt gtatcatatg 180
ccaagtacgc cccctattga cgtcaatgac ggtaaatggc ccgcctggca ttatgcccag 240
tacatgacct tatgggactt tcctacttgg cagtacatct acgtattagt catcgtatt 300
accatggtga tgcggttttg gcagtacatc aatgggcgtg gatagcggtt tgactcacgg 360
ggatttccaa gtctccaccc cattgacgtc aatgggagtt tgttttgaat gaaagacccc 420
acctgtaggt ttggcaagct agcttaagta acgccatttg caaggcatgg aaaaatacat 480
aactgagaat agagaagttc agatcaaggc caggaacaga gaaacaggag aatatgggcc 540
aaacaggata tctgtggtaa gcagttcctg ccccgctcag ggccaagaac agttggaaca 600
ggagaatatg ggccaacag gatattctgt gtaagcagtt cctgccccgc tcagggccaa 660
gaacagatgg tcccagatc ggtcccgcc tcagcagttt ctagagaacc atcagatgtt 720
tccaggtgc cccaaggacc tgaaatgacc ctgtgcctta tttgaactaa ccaatcagtt 780
cgcttctcgc ttctgttcgc gcgttctgc tccccgagct caataaaaga gccacaacc 840
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<210> 70
<211> 455
<212> DNA
<213> Artificial Sequence

<220>
<223> MPSV LTR promoter without the negative control
region.

<400> 70
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aatatgggcc aaacaggata tctgtggtaa gcagttcctg ccccggtca gggccaagaa 120
cagatggtcc ccagatgctg tcccgcctc agcagtttct agagaaccat cagatgtttc 180
cagggtgccc caaggacctg aaaatgacct tgtgccttat ttgaactaac caatcagttc 240
gcttctcgct tctgttcgct cgcttctgct ccccgagctc aataaaagag cccacaaccc 300
ctcactcggc gcgccagtcc tccgatagac tgcgtcgccc gggtagccgt gttctcaata 360
aacctcttg cagttgcctc cgactcgtgg tctcgtgtt ccttgggagg gtctcctctg 420
agtgattgac taccgctcag cgggggtctt tcagt 455